THE EFFECT OF HEAT UPON THE BIOLOGICAL VALUE OF MEAT PROTEIN

AGNES FAY MORGAN AND GRACE E. KERN
Laboratory of Household Science, University of California, Berkeley

(Received for publication July 11, 1933)

Work previously reported from this laboratory (Morgan, '31) showed that cereal proteins and casein subjected to dry heat or toasting were not so well utilized for growth and did not allow such favorable nitrogen balances as did the raw cereal and casein. Since the digestibility of the toasted proteins was but little different from that of the raw, the difference in the value of the two could not be attributed to this source and so must have been due to the detrimental action of the heat on metabolic availability.

Ingvaldsen ('29), Daniel and McCollum ('29), Maynard and Tunison ('32), Schneider ('32) and Maynard, Bender and McCay ('32) have all conducted experiments on fish meals which were dried in various ways and have found that a high temperature used in the drying process was more detrimental to the nutritive value of the fish protein than a lower one. In a recent report, Fixsen and Jackson ('32) have confirmed the finding that casein suffers a marked decline in biological value as the result of prolonged heating even at 112°C.

Since meat is such an important item of food in our present diets, it was thought that a similar study of the effect of various methods of preparation on the nutritive value of meat would be profitable. A large number of figures are available giving the protein value of different meats and edible animal organs, but little is at hand concerning the effect of cooking on these figures. When work was begun on

this experiment, there were available the results of the work of Jarussowa ('29) and the first work by Scheunert and Bischoff ('30). The latter workers performed growth and reproduction experiments to determine the nutritive value of a pure meat diet prepared from raw, boiled or autoclaved meat with no addition other than minerals and vitamins, and concluded that there were no significant differences among the three different groups. These investigators realized that this work was open to criticism because the high protein which they fed might compensate to some extent for deficiencies and make it impossible to detect injuries which may have been caused by the heating. Therefore, Scheunert and Venus ('32) repeated the work, using both fresh and frozen meat, raw, boiled and fried, at a 10 per cent level in the diet. The results of this experiment, which were published after the present study was planned, again showed no significant differences among any of the meats used. Scheunert and Venus, however, did not use autoclaved meat.

The work of Jarussowa ('29) consisted in feeding diets containing ground meat which had been left raw or treated in one of the following ways: 1) boiled in water from 20 to 25 minutes; 2) boiled in a tightly covered casserole in a special salt bath for 4 hours; 3) kept in a thermos for 5 hours at a temperature from 75° at the beginning to 66° at the end. The results showed no difference in biological value. Since Jarussowa mixed 100 gm. of meat, treated in one of the ways given above, with 40 gm. of potatoes, 20 gm. of cabbage and 10 gm. of carrots, a mixed rather than a meat protein was studied.

The meat used in this experiment was bottom round of beef which was freed from connective tissue and fat and cut into 2-inch cubes. These were divided equally into four portions which were prepared in different ways. The first portion was ground raw three times and then spread out into very thin layers to dry. The second portion of meat cubes was boiled in single layers in water until the internal temperature was 84°C. This temperature was selected because previous work in this laboratory had shown that meat is thoroughly

cooked when this point is reached. The third portion of the meat was cooked in single layers in an autoclave at 15 pounds' pressure for 7 minutes. This gave the same internal temperature of 84°C. which was used in the case of the boiled meat. The last portion was autoclaved at 15 pounds' pressure for 1 hour. All the meat portions were ground to a fine powder when thoroughly dried. The water extract or broth from the cooked preparations was saved and dried along with the meat from which it was obtained.

TABLE 1

Daily endogenous nitrogen metabolism of adult male rats (on low egg diet)

NUMBER OF RATS	NUMBER OF PERIODS	AVERAGE BODY WEIGHT	N INTAKE	URINARY N	FECAL N	BODY N IN URINE PER 100 GM. WEIGHT	BODY N IN FECES PER GRAM FOOD EATEN
		gm.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	4	262	96	42	25	16	1.7
2	4	264	95	47	33	18	2.2
3	4	312	113	58	33	18	1.7
4	4	296	98	54	26	19	1.7
5	4	284	98	48	26	18	1.7
6	4	310	110	57	30	18	1.7

Two types of tests were made with these four portions of meat, that is, growth and balance experiments. The diets were made up as follows:

	Per cent
Dried beef	8.5 (1.07 to 1.16 per cent N)
Agar	4.0
Salt mixture	4.0 (Osborne & Mendel)
Hydrogenated vegetable fat[1]	10.0
Cornstarch	73.5

[1] Crisco.

Two drops of cod liver oil and 75 mg. of a yeast extract (containing 5 mg. N) were given separately daily to each animal to supply the necessary vitamins.

Rats were placed on these diets at 28 days of age and their food intakes and body weight increases noted for the 6 weeks following. The results of these observations are shown in table 3.

Six adult male rats were used for the balance experiments which were carried out in the manner previously reported (Morgan, '31). Four 5-day periods on the same diet with dried whole egg substituted for the meat were included among these balances, two periods at the beginning and two near the end of the tests. The nitrogen excretion in these periods was looked upon as representing the endogenous level, following the reasoning of Mitchell and Carman ('26). Data on these periods are shown in table 1. Two balances were obtained for each rat on each of the four meat diets and biological values calculated from the results as shown in table 2.

DISCUSSION

It is clear that there is a decrease in nutritive value of the beef protein caused by all three heating processes used. This is greatest in the meat autoclaved for 1 hour at 15 pounds' pressure and about the same in the other two cooked samples. The latter samples, boiled and autoclaved 7 minutes at 15 pounds' pressure, were cooked to the same internal temperature, 85°C., but this was probably exceeded in the sample autoclaved for 1 hour. The biological values (table 2) obtained by the balance sheet method on adult rats for the raw and autoclaved 1 hour samples differ by 11 ± 2.2, while the raw and boiled differ by 7 ± 1.8 and the raw and autoclaved 7 minutes by 5 ± 2.1. The significance of the first mentioned difference is obvious. The injury to the protein appears to be gradual and progressive with more prolonged and severe heating.

By the criterion of growth promoted in young rats (table 3) quite parallel differences are deduced. The raw meat is superior to all the cooked products, since each gram of raw meat protein eaten produced 0.78 ± 0.07 gm. greater gain than did that autoclaved 1 hour, 0.17 ± 0.06 more than the boiled and 0.14 ± 0.06 more than that autoclaved 7 minutes. The differences are in the same order and significant in nearly the same degree as are those shown by the balance experiments. Since the protein intake of all four groups was nearly

TABLE 2

Daily metabolic data showing biological value of beef, raw and cooked in three different ways

PREPARATION OF MEAT	RAT NUMBER	BODY WEIGHT	N INTAKE	URINARY N	FECAL N	ENDOGENOUS N		FOOD N ABSORBED	FOOD N RETAINED	BIOLOGICAL VALUE	AVERAGE
						Urine	Feces				
		gm.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*	*per cent*
Raw	1	252	152	103	25	44	23	150	91	61	
		253	163	109	29	44	24	158	93	59	
	2	260	169	100	36	54	35	168	122	72	
		262	184	105	37	55	38	184	134	73	
	3	275	157	94	27	54	23	153	113	73	
		274	140	92	25	54	21	136	98	72	
	4	271	153	109	27	53	23	149	93	62	
		317	176	122	35	55	26	167	100	60	
	5	265	124	92	22	56	19	121	85	70	
		263	155	103	22	55	23	155	107	69	
	6	304	189	121	28	68	27	188	125	66	
		356	208	127	48	63	33	193	129	67	67±1.5
Boiled at ordinary pressure	1	252	134	102	23	44	21	132	74	56	
		249	141	107	27	43	23	137	73	53	
	2	246	159	109	35	52	34	158	101	64	
		248	170	115	41	52	36	165	102	62	
	3	278	127	106	23	55	20	124	73	59	
		276	150	109	29	54	24	145	90	62	
	4	272	162	116	27	53	26	161	98	61	
		272	178	127	29	53	28	177	103	58	
	5	267	158	122	26	56	25	157	91	58	
		348	209	131	32	48	30	207	124	59	
	6	289	166	112	25	55	25	166	109	65	
		294	187	123	31	56	28	184	117	63	60±0.9
Autoclaved 7 minutes at 15 pounds pressure	1	290	158	119	27	42	23	154	77	50	
		256	157	113	29	44	23	153	85	55	
	2	316	161	110	31	49	30	160	90	62	
		268	159	110	35	56	35	159	104	65	
	3	362	138	106	25	59	21	134	87	65	
		274	206	123	33	54	32	205	136	66	
	4	319	196	124	31	55	30	195	126	65	
		273	165	112	26	53	26	665	106	64	
	5	270	161	108	25	57	25	161	110	68	
		272	139	107	21	57	22	139	89	64	
	6	346	189	135	30	61	30	189	115	61	
		313	177	127	31	59	26	174	105	60	62±1.4
Autoclaved 1 hour at 15 pounds pressure	1	257	151	121	26	45	24	149	73	49	
		282	140	112	24	41	21	137	66	48	
	2	302	161	104	34	47	32	159	102	64	
		277	173	119	41	58	38	170	109	64	
	3	372	128	109	27	61	21	122	74	60	
		380	131	112	26	62	21	126	76	60	
	4	286	191	142	36	56	30	185	99	53	
		290	190	141	32	56	30	188	103	54	
	5	270	153	132	23	57	24	153	78	51	
		272	163	132	25	57	26	163	88	54	
	6	328	156	121	26	58	27	156	93	61	
		315	193	136	32	60	29	190	114	59	56±1.6

the same, the growth differences observed may be fairly ascribed to variations in the nutritive value of the meat protein preparations. The actual total food intake of the group on raw meat diet was somewhat larger than in the other three groups but since the protein content of this diet was slightly lower than that of the others the nitrogen intake remained the same as in the other groups. There may, nevertheless, be some advantage gained for the raw meat diet through the larger average caloric intake. Both total and protein intakes, however, were nearly identical in the three groups fed the cooked meat diets but growth response in the animals given the meat autoclaved 1 hour was significantly

TABLE 3

Growth of rats during 6 weeks on diets containing 8.5 per cent dried beef (7 per cent protein) raw and cooked in three different ways

PREPARATION OF MEAT	PROTEIN CONTENT OF DIET	NUMBER OF RATS	AVERAGE BODY WEIGHTS			TOTAL PROTEIN INTAKE	TOTAL FOOD EATEN	GAIN IN BODY WEIGHT PER GRAM PROTEIN EATEN
			Initial	Final	Gain			
	per cent		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Raw	6.7	17	58	126	68	26.4	394	2.58±0.04
Boiled	7.1	18	58	117	59	24.5	345	2.41±0.06
Autoclaved 7 minutes	7.1	18	57	118	61	25.0	352	2.44±0.06
Autoclaved 1 hour	7.3	18	58	104	46	26.0	356	180±0.05

lower than in the others. All of the conditions required for definite establishment of inferiority of the latter diet appear to be fulfilled in the three cooked meat diets at least.

The digestibility data shown in table 2 indicate that the decrease in value of the cooked meat proteins is not due to a lowering in absorption since the digestibility coefficient of the raw beef protein is 97 and that of the cooked samples, 98, 99 and 98. The same observation was made in experiments upon heated gluten and casein previously reported (Morgan, '31).

An interesting difference between the biological value of meat proteins for maintenance and for growth when fed at this low level, 7 per cent, is seen in these data. The growth method of Osborne, Mendel and Ferry ('16) indicates a high

classification for beef nitrogen. Casein fed at this level, 7 per cent, produces almost no growth, while the raw beef produced nearly normal growth. Cereal proteins were found by Osborne and Mendel ('20) to afford gains of 1.4 to 1.8 gm. per gram of protein eaten at 5 to 10 per cent levels for 4-week periods, and casein 1.45 at 9.3 per cent level (Osborne and Mendel, '16). Wheat gluten was found by Morgan ('31) to allow growth for 8 weeks at the rate of 1.3 gm. per gram of protein eaten at 7 to 15 per cent levels. Hoagland and Snider ('26 a), using a 10 per cent level and 60-day period, report 2.37 for beef, 2.24 for pork, 2.21 for mutton and quite similar figures for the corresponding hearts, livers and kidneys. The experiments here reported show a maximum of 2.58 at 7 per cent level for raw beef round. Milk proteins at 10 per cent level were found by Hoagland and Snider ('26) to allow 2.36 gm. gain per gram of protein eaten in 60 days. Wheat, oatmeal and beans, under similar conditions, yielded 1.58, 1.89 and 1.32 gm. gain per gram of protein eaten.

The objection may be raised that the total amounts of food and of protein eaten may be widely different on these different diets and that the amounts of protein available for growth may be affected thereby. Some of these data have been brought together in table 4 in order to discover whether this objection is valid. It will be noted that no consistent differences can be clearly ascribed to the rather small variations in intake. Thus a whole wheat diet eaten at the rate of 10.9 and 10.1 gm. per day allows growth of 1.2 and 1.68 gm. per gram of protein, whereas with intake of 7.4 gm. per day the figure is 1.58 gm. per gram of protein eaten. The conditions used for the two latter tests, however, are closely comparable with each other but not with the first experiment. Certainly both total and protein intakes recorded for the cereals by Osborne and Mendel ('20) are comparable with those of Hoagland and Snider ('26, '26 a) and with those here reported for meat and yet the growth rates recorded are significantly different for the two groups of proteins.

The biological values obtained by nitrogen balance studies obtained usually upon adult rats for the same proteins diverge from these figures. Corn and oat proteins at 5 per cent level have biological values of 72 and 79 (Mitchell, '24), wheat gluten 83, (Morgan, '31), wheat 72 (Mattill and Clayton, '30), casein 71 and 66 (Mitchell, '24; Morgan, '31). Beef round protein at 8 to 10 per cent level has a biological value of 69 (Mitchell and Carman, '26) and at 7 per cent level, according to the data here reported, of 67. All of these values lie in the same plane in contrast with the considerably larger growth figures quoted above for the meat proteins as compared with

TABLE 4

Comparison of growth values of certain proteins

PROTEIN	LEVEL	AVERAGE DAILY INTAKE		GROWTH PER GRAM PROTEIN EATEN	FEEDING PERIOD	AUTHORS
		Food	Protein			
	per cent	*gm.*	*gm.*	*gm.*	*days*	
Wheat	8	10.9	0.87	1.2	28	Osborne & Mendel ('20)
Rye	8	10.7	0.85	1.5	28	Osborne & Mendel ('20)
Oats	8	8.3	0.66	1.4	28	Osborne & Mendel ('20)
Barley	8	12.8	1.02	1.8	28	Osborne & Mendel ('20)
Casein	9.3	6.9	0.61	1.45	28	Osborne, Mendel & Ferry (16)
Casein	8.0	5.7	0.49	1.36	35	Morgan, A. F. (unpublished data)
Wheat gluten	7– 9	6.2	0.56	1.31	56	Morgan ('31)
Wheat gluten	10–12	6.0	0.75	1.29	56	Morgan ('31)
Wheat	10	10.1	1.01	1.68	56	Morgan ('31)
Wheat	10	7.4	0.74	1.58	60	Hoagland & Snider ('27)
Oatmeal	10	7.0	0.70	1.89	60	Hoagland & Snider ('27)
Beans	10	6.6	0.66	1.32	60	Hoagland & Snider ('27)
Milk	10	8.6	0.86	2.36	60	Hoagland & Snider ('26, '26a)
Beef	10	9.7	0.97	2.37	60	Hoagland & Snider ('26, '26a)
Pork	10	9.0	0.90	2.24	60	Hoagland & Snider ('26, '26a)
Mutton	10	9.5	0.95	2.21	60	Hoagland & Snider ('26, '26a)
Beef, raw	7	9.3	0.65	2.58	42	This report
Beef, boiled	7	8.2	0.57	2.41	42	This report
Beef, autoclaved 7 min.	7	8.3	0.58	2.44	42	This report
Beef, autoclaved 1 hour	7	8.4	0.59	1.80	42	This report

cereal proteins and casein. Such a discrepancy is not observable in the values for maintenance and growth of milk and egg proteins. Biological values for these at 8 to 10 per cent levels are 94 and 85 (Mitchell and Carman, '26). At 5 per cent level, Mattill and Clayton ('30) found 89 for milk, Mitchell ('24), 93. The growth value for milk is given by Hoagland and Snider ('26) as 2.36 gm. gain per gram of protein eaten. Comparable growth data for whole egg proteins appear not to be available.

It may be fair to state then that weight gains of 2 to 3 gm. per gram of food protein eaten are made by young rats when proteins of high biological value, such as meat, milk or eggs, are fed at 5 to 10 per cent level and that the gains are only 1 to 2 gm. per gram of food protein eaten when casein and cereal grains are fed. The corresponding biological values at 5 to 10 per cent levels for maintenance of adult rats are 93 and 85 or 89 for eggs and milk, 67 to 69 for beef and 60 to 70 for casein and cereal grains. Thus beef protein falls in the higher group for growth but the lower for maintenance. It should be noted, however, that the value 69 for beef was obtained by Mitchell and Carman ('26) in experiments upon young growing rats.

An explanation for this curious discrepancy is not obvious, since the reverse condition might ordinarily be expected. The theory of McCollum and Steenbock ('12) of a qualitative difference between tissue protein synthesis and repair may be recalled in this connection. It is possible that the chief demands for amino acids in adult maintenance are quite different from those involved in the growth of tissues. The synthesis of proteins of the digestive juices and of the internal secretions may make up the major needs in the adult and the value of meat muscle proteins for these purposes may be markedly lower than for tissue growth. The reported inferiority (Nelson, Irwin and Peet, '30) of beef muscle for the support of lactation favors this suggestion.

These findings, if confirmed, may point to the validity of the popular impression that meat is less valuable to, and less

well tolerated by, persons in middle and old age than by growing children. Further studies are needed, however, applying the criterion of analyzed body weight increases to adult and the balance method to young growing animals.

EXPERIMENT WITH HORSE MEAT

A large sample of horse meat, lean round, became available[1] soon after the beef studies were completed and was used for two series of similar growth experiments. The rats ate this diet so well that it was necessary to reduce the protein content to 5 per cent in order to obtain intakes comparable with those of the beef experiment.

TABLE 5

Growth of rats during 6 weeks on diets containing dried horse meat

PREPARATION	PROTEIN CONTENT OF DIET	NUMBER OF RATS	AVERAGE BODY WEIGHTS			TOTAL PROTEIN INTAKE	TOTAL FOOD EATEN	GAIN IN BODY WEIGHT PER GRAM OF PROTEIN EATEN
			Initial	Final	Gain			
	per cent		*gm.*	*gm.*	*gm.*			
Raw	5.0	15	52	127	75	25.3	507	2.96
Raw	6.7	9	48	134	86	32.1	473	2.68
Autoclaved 1 hour	5.4	15	51	112	61	24.6	456	2.48
Autoclaved 1 hour	6.8	9	48	106	58	26.2	486	2.21

The meat had been ground immediately after cutting and a portion was at once dried at low temperature to be used as the raw sample. Another portion was autoclaved for 1 hour at 15 pounds' pressure. Because of the fineness of division it was difficult to obtain uniform penetration of heat during the autoclaving even though the meat was separated by tying it up in small cheese-cloth bags.

As is shown in table 5 the total food intakes were in all cases larger than were those of the beef-fed rats although the protein intakes were nearly the same as in the beef series

[1] Through the courtesy of Dr. C. M. Haring of the Division of Veterinary Science, University of California.

except for the 6.7 per cent raw horse meat diet.[2] Nevertheless, the gains per gram of horse meat protein were larger than in the beef series, 2.96 and 2.48 gm. gain per gram of raw and autoclaved horse meat protein eaten on total protein intakes similar to those yielding 2.58 and 1.80, in the beef experiments. However, if level of protein fed be considered the gains on 6.8 per cent horseflesh were quite comparable with those on 6.7 per cent beef, 2.68 and 2.58. The higher value seen in the autoclaved horse meat series, 2.21 as compared with 1.80 may be due to the less efficient subdivision of the meat during autoclaving.

These results on horse meat are offered chiefly for their possible value in current discussions of dog rations.

SUMMARY

1. The biological value for maintenance of raw beef muscle protein at 7 per cent level tested on rats was found to be greater than that of the same meat cooked in three different ways. The figure for the raw beef is 67, for that boiled at ordinary pressure, 60 (to internal temperature of 85°C.), boiled for 7 minutes at 15 pounds' pressure, 62 (to internal temperature of 85°C.), boiled for 1 hour at 15 pounds' pressure, 56. There appears to occur a heat injury to the protein increasing in severity with the length of exposure and the height of the temperature reached.

2. Young rats were fed these rations for 6 weeks and were found to grow best on the raw beef diet. The gain in body weight per gram of protein eaten was 2.58 ± 0.04 on the raw beef, 2.41 ± 0.06 and 2.44 ± 0.06 on the boiled and autoclaved 7 minutes' preparations and 1.80 ± 0.05 on that autoclaved 1 hour. The food intake was slightly larger on the raw diet than on the other three but not sufficiently to account for the extra growth seen.

[2] The assistance of Louise Kimmel in this part of the experiment is acknowledged gratefully.

3. Horseflesh prepared raw and autoclaved for 1 hour by similar methods was fed at 5 and 6.8 per cent levels in the same basal diet to young rats. The total intakes on these diets were in all cases considerably larger than on the beef diets but at a comparable level the gain in weight of the rats in 6 weeks per gram of raw horseflesh protein eaten was similar to that for beef, 2.68. The autoclaved horseflesh yielded a higher value than the similar beef, 2.21 as compared with 1.80, due perhaps to poorer heat penetration in the former.

4. Attention is drawn to the discrepancy between the high values for growth of the beef protein, parallel with the best values obtained on other animal proteins, and the lower values for maintenance shown by the biological values, parallel with casein and the cereal proteins. This may be due to sharp differences between the mechanisms of the endogenous protein metabolism of growth and maintenance.

LITERATURE CITED

CLAYTON, M. M. 1930 The comparative value of different food proteins for reproduction and lactation in the rat. I. Beef muscle, liver and kidney. J. Nutrition, vol. 2, p. 491.

DANIEL, E. P., AND E. V. MCCOLLUM 1931 Studies on the nutritive values of fish meals. U. S. Dept. Com., Bur. Fisheries Invest. Rpt. 2, vol. 1.

FIXSEN, M. A. B., AND H. M. JACKSON 1932 The biological values of proteins. IV. The biological values of the proteins of wheat, maize and milk. Biochem. J., vol. 26, p. 1923.

HOAGLAND, R., AND G. C. SNIDER 1926 Nutritive value of the protein in veal and calf sweetbreads; in beef cheek meat, lips, tongues, brains, spleen, and tripe; and in hog brains and tongues. J. Agr. Res., vol. 32, p. 679.

———— 1926 a Nutritive value of the protein in voluntary muscle, heart, liver and kidney from cattle, sheep and hogs. J. Agr. Res., vol. 32, p. 1025.

———— 1927 The value of beef protein as a supplement to the proteins in certain vegetable products. J. Agr. Res., vol. 34, p. 297.

INGVALDSEN, T. 1929 Fish meals. Part I. The effect of the high temperature employed for drying, on the nitrogen partition in fish meals. Canad. Chem. Metall., vol. 13, p. 97.

JARUSSOWA, N. 1929 Der Einfluss des Kochens auf den Nahrwert der Nahrung. Biochem. Zeit., Bd. 207, S. 395.

MATTILL, H. A., AND M. M. CLAYTON 1930 The nutritive value of cereal breakfast foods. V. The biological value of some cereal proteins as determined on rats. J. Nutrition, vol. 3, p. 17.

MAYNARD, L. A., R. C. BENDER, AND C. M. MCCAY 1932 Vitamin A and protein content of various fish meals. J. Agr. Res., vol. 44, p. 591.

MAYNARD, L. A., AND A. V. TUNISON 1932 Influence of drying temperature upon digestibility and biological value of fish proteins. Ind. Eng. Chem., vol. 24, p. 1168.

MCCOLLUM, E. V., AND H. STEENBOCK 1912 Studies of the nutrition of the pig. Notes on the repair processes of protein metabolism. Wis. Agri. Exp. Sta. Res. Bull. no. 21, p. 58.

MITCHELL, H. H. 1924 A method of determining the biological value of protein. J. Biol. Chem., vol. 58, pp. 873, 905.

MITCHELL, H. H., AND G. G. CARMAN 1926 The biological value of the nitrogen of mixtures of patent white flour and animal foods. J. Biol. Chem., vol. 68, p. 183.

MORGAN, A. F. 1931 The effect of heat upon the biological value of cereal proteins and casein. J. Biol. Chem., vol. 90, p. 771.

NELSON, P. M., M. H. IRWIN, AND L. J. PEET 1930 Meat in nutrition. I. Preliminary report on beef muscle. J. Nutrition, vol. 3, p. 303.

OSBORNE, T. B., AND L. B. MENDEL 1920 Nutritive value of the proteins of the barley, oat, rye and wheat kernels. J. Biol. Chem., vol. 41, p. 275.

OSBORNE, T. B., L. B. MENDEL, AND E. L. FERRY 1916 A method of expressing numerically the growth-promoting value of proteins. J. Biol. Chem., vol. 37, p. 223.

SCHEUNERT, A., AND H. BISCHOFF 1930 Über den Nahrwert reiner Fleischkost, hergestellt aus rohem, gekochtem und autoklaviertem Muskelfleisch bei Ratten. Biochem. Zeit., Bd. 219, S. 186.

SCHEUNERT, A., AND C. VENUS 1932 Über den Nahrwert des Muskelfleisches für Wachstum und Fortpflanzung. Biochem. Zeit., Bd. 252, S. 231.

SCHNEIDER, B. H. 1932 Nitrogen balance studies with various fish meals. J. Agr. Res., vol. 44, p. 723.

THE INFLUENCE OF ROUGHAGE ON PROTEIN DIGESTIBILITY

WILLIAM H. ADOLPH AND MAO-YI WU
Department of Chemistry, Yenching University, Peiping, China

(Received for publication July 14, 1933)

It is not unusual in metabolism experiments in China and India to find coefficients of protein utilization in mixed diets as low as 40 and 50 per cent. This low figure must mainly be due to the use of vegetable proteins of intrinsically low digestibility and low biological value, but the question also is raised whether it may not be due in part to the bulk of the diet. In China, materials of lesser nutritive value, such as chaff and even clay, are included in the diet during famine or times of failing food supply. A lowered degree of utilization, resulting in an appreciable loss of protein material, might be of pronounced economic importance in a country where food protein is a limiting factor in nutrition.

The classical experiments of Rubner ('18) demonstrated that the ingestion of foods which contain fibrous cellular substance tends to cause low values for protein digestibility. Whitacre, Willard and Blunt ('29) more recently report a lowering of protein digestibility in man due to fiber in the diet. Weinert ('31), however, fed to sheep a ration containing 31 per cent of quartz sand, and found no marked effect on the degree of nitrogen utilization.

The following experiments with rats and with human subjects were designed to secure data on the manner in which the degree of digestibility of food protein is affected by the inclusion of exceptionally large amounts of roughage bulk in the diet.

EXPERIMENTS AND OBSERVATIONS

Experiment 1. The effect of varying the total amount of food on the digestibility of rice in rats. Two rats were placed in separate galvanized wire cages with false bottoms. The cages rested in glazed dishes in which were spread layers of filter paper so that feces might not be contaminated with urine. Steamed rice was fed in varying amounts over three periods, the amount per day being held constant for each period of 6 days. Rice was chosen because it has a high coefficient of digestibility and because it could be prepared in a semi-pasty form, not readily scattered by the rats. Vitamins were supplied by a daily dose of 5 drops of cod liver oil and 0.5 gm. of yeast for each rat. The feces were collected over exact 72-hour intervals, this interval representing the last 3 days of each period; no marker was used. The food was cooked with the same amount of water once every day, and the intake recorded on the dry basis. In the first period each rat was given as much as it desired; this was then reduced to two-thirds and finally to one-third for the succeeding periods. The rice in the second and third periods was fed twice daily so that the rats might not eat the entire amount at once. Nitrogen determinations were made by the Kjeldahl method and the digestibility calculated from the formula:

$$\text{Digestibility} = \frac{\text{Food N} - (\text{fecal N} - \text{metabolic N})}{\text{food N}}$$

The results shown in table 1 indicate that varying the daily amount of nutrient food does not alter the degree of digestibility.

Experiment 2. The effect of varying the proportion of added bulk on the digestibility of rice in rats. This was carried out with rats in the same manner as in the preceding experiment, except that while rice was used as the food material, roughage material was added in gradually increasing proportions. Each dietary period was in most cases 6 days in length, metabolism measurements being confined to the last 3 days. Toward the end of each series when some of the animals at high roughage levels began to lose appetite, it was found expedient to decrease each period to 5 days.

Four different varieties of roughage materials were used: filter paper, agar agar, China clay, and rice chaff. The China clay, secured from Kiukiang, Kiangsi, was white in color and a good grade of kaolin. In famine years the very poor peasants eat this clay in large amounts with rice or wheat and a green vegetable; it is called by them Kwan Yin Fen (Goddess of Mercy flour), being looked upon as a gift from the goddess to the poor. The rice chaff is also a substance added to the food in times of famine in many parts of China. The alimentary tract of vegetarians becomes habituated to a large bulk, and its demand for satisfaction in terms of large volume of food is very real.

TABLE 1

Effect of varying the total amount of intake on the protein digestibility of rice in rats (for 3-day periods)

RAT NO.	PERIOD	BODY WEIGHT, GM.	INTAKE		FECAL NITROGEN, MG.	DIGESTIBILITY[1]
			Rice, Gm.	Nitrogen, Mg.		
1025	1	60	31.4	482	55.4	97.7
	2	61	19.0	292	33.4	97.6
	3	53	9.6	147	16.4	98.0
1026	1	55	24.8	380	46.1	97.1
	2	56	13.3	204	26.5	96.0
	3	49	8.0	123	12.2	99.2

[1] Metabolic nitrogen is estimated at 1.4 mg. per gram of food intake; see experiment 2.

The filter paper and kaolin were nitrogen-free, the agar agar was almost nitrogen-free (0.2 per cent of nitrogen, dry basis), while the rice chaff had a nitrogen content equal to that of rice. The nitrogen of rice chaff is very poorly utilized in the alimentary tract; preliminary determinations indicated that its digestibility was 40 per cent, and allowance was made accordingly in calculating the digestibility figures for this ration. The roughage materials in each case were mixed with the rice during cooking, special care being taken to secure an intimate mixture. Non-scattering food cups were used. The food was fed in all cases ad libitum; intake and level of the bulk material are all recorded on a dry basis. Fecal

nitrogen was determined in the composite 3-day sample. A small amount of greens was given at the beginning of each period, and vitamins were supplied by feeding yeast and cod liver oil as above in uniform daily doses. The nitrogen of the yeast was neglected in the calculations.

TABLE 2

Effect of varying the level of roughage intake on the protein digestibility of rice in rats. (Averages per rat per day; 3-day periods except as noted; three rats in each group)

	TOTAL FOOD INTAKE, GM.	NITROGEN INTAKE, MG.	FECAL NITROGEN, MG.	DIGESTIBILITY[2]	TOTAL FOOD INTAKE, GM.	NITROGEN INTAKE, MG.	FECAL NITROGEN, MG.	DIGESTIBILITY[2]
Roughage material	Filter paper				Rice chaff[3]			
Per cent								
0	12.0	186	37	90	18.6	286	67	86
5	10.1	147	26	92	18.4	283	65	87
10	10.1	139	29	89	22.4	344	88	88
20	9.9	122	25	91[1]	18.3	281	57	87[1]
0	17.7	272	56	89[1]	23.5	360	102	81[1]
Roughage material	Clay				Agar agar			
Per cent								
0	22.0	341	69	89	12.5	193	42	87
5	22.0	321	71	88	10.4	152	39	84
10	21.8	300	68	88	10.1	139	40	81
20	24.0	295	56	92[1]	6.4	78	27	75
0	24.6	379	76	89[1]	12.9	198	46	86
40	26.9	247	58	91[1]	8.5	78	39	66[1]
60	35.0	214	57	96[1]	—	—	—	—
80	54.7	169	101	86[1]		—	—	—

[1] Two-day periods.

[2] Metabolic nitrogen reckoned at 1.4 mg. per gram of food intake.

[3] Nitrogen content = 1.53 per cent; digestibility of rice chaff reckoned at 40 per cent.

The summarized results are shown in table 2. Digestibility was calculated by the above-mentioned formula. In all cases the animals, after being carried through several stages of increasing roughage intake, were again returned to a zero level. This served to demonstrate that the basic level was definite and unchanging, and also that the effects produced

were definitely related to the addition of roughage material. It was not possible to increase the proportion of roughage material to the same degree in all four cases, inasmuch as the rats refused to consume certain of the food mixtures when the per cent of roughage was increased to the highest levels. The diets most highly relished by the rats were the clay-rice diets. Filter paper tended to cause digestive difficulties; one rat died of intestinal obstruction on a 40 per cent filter paper intake. The rats employed weighed from 75 to 150 gm.; there were three rats in each of the four groups. The growth records of the rats are not regarded as significant and are not reproduced here; growth of all animals was satisfactory except where the roughage intake reached a level of 50 per cent or over. Frey, Harding and Helmbold ('28) obtained excellent growth with rats fed diets containing 25 per cent of rice cellulose.

One of the complicating factors in the calculation of digestibility is the metabolic nitrogen. For each of the rats used in this experiment, we determined metabolic nitrogen by feeding a nitrogen-free diet of cornstarch only, with the usual amounts of vitamins. The values obtained ranged from 1.15 to 1.70 mg., with an average of 1.43 mg. of nitrogen per gram of food intake. Mitchell ('24) concludes from his experiments that the amount of fecal nitrogen of endogenous origin is proportional to the weight of dry food ingested, and we have assumed that this is true. He also notes that this metabolic nitrogen coefficient increases when filter paper is added to the diet. Our observations confirm this, but the coefficient does not seem to vary directly as the amount of roughage added. Boas Fixsen and Jackson ('32) indicate that, with adult rats at least, the actual amount of metabolic nitrogen in the feces is a constant for each individual rat irrespective of the intake. But, whatever the correct manner of representing this factor, it is apparent that with a high nitrogen intake the correction for nitrogen of endogenous origin becomes less significant. It was decided therefore to employ the single figure, 1.4 mg., to express the metabolic nitrogen per gram of food intake for

all intake levels throughout experiments 1 and 2. Introducing into the calculations a figure for metabolic nitrogen slightly higher for each increasing roughage level, it is seen, would tend to give slightly higher rather than lower values for the percentage digestibility.

In table 3 are recorded the average dry weights of feces per gram of intake for the roughage-containing diets at 0, 5, and 10 per cent levels, collected over the 3-day metabolism period. The feces were all well formed. Measurements made with these rats, using carmine markers, showed that the agar agar diets required from 5 to 7 hours to pass through the digestive tract, while the other three diets required 9 to 12

TABLE 3

Average dry weight of feces of rats fed different bulk materials; totals for 3-day periods (in grams per gram of dry food intake)

		ROUGHAGE MATERIAL			
		Filter paper	Agar agar	Clay	Rice chaff
	NUMBER OF RATS	3	3	3	3
	Per cent				
Level of roughage intake	0	0.043	0.042	0.042	0.048
	5	0.103	0.112	0.082	0.065
	10	0.146	0.169	0.133	0.092

hours. The diet of rice only, without roughage, also required 9 to 12 hours. Agar agar possessed outstanding laxative properties, not only causing the material to pass through the alimentary tract with marked rapidity but also producing softer feces. In none of the other cases did the added roughage material appear to change either the character of the feces or the frequency of defecation. From table 3 it may be noted that the increase of fecal weight with the rats fed clay and also those fed filter paper was practically equal to that of the roughage material fed. Agar agar, however, definitely caused an increase in dry fecal weight; in other words it acted as a feces former.

Experiment 3. The effect of the addition of roughage bulk material on the digestibility of a meat-rice diet in man. In this experiment, instead of feeding pure filter paper cellulose, we employed cellulose prepared from cabbage, using human subjects. Two Chinese, accustomed to the monotonous cabbage-cereal diet of north China, both 20 years of age and about 55 kilos body weight, were fed a meat-rice diet to which was added cabbage fiber in amounts approximating and even exceeding the large bulk of cabbage to which the north China peasant is accustomed. The cabbage fiber was prepared by cooking the stems of cabbage with water until very soft. The material was then strained in cloth bags, and pressed free from water. It was then cooked again, with fresh water, strained and pressed again, and finally sun-dried. The product represented 2 per cent by weight of the original cabbage; the 150 gm. of this dry fiber fed during the second period was therefore equivalent to about 7.5 kilos of fresh cabbage, or 2.5 kilos for each day—a very considerable amount. The cabbage fiber used in the third period received the same treatment except that the final drying was omitted. These two preparations contained 1.40 and 1.33 per cent of nitrogen respectively, and showed a crude fiber content of 50 per cent, all calculated on a dry basis.

The diets fed are shown in table 4; the protein was supplied by lean meat and rice. The subjects ate three meals a day; the morning meal was a light one, consisting of lotus flour gruel; the meat and rice were cooked together, and, with cabbage fiber during the experimental periods, were consumed at noon and in the evening. The common Chinese diet is simple, easily prepared, and readily adapts itself to metabolism studies. The intake for both subjects was identical, except that water and salt were taken ad libitum. The subjects ate and lived in the laboratory. Each of the three feeding periods was 6 to 7 days in length, and succeeded each other without interval. Markers were used for the feces which were collected for the last 3 days of each period; aliquots were taken for analysis of the well-mixed 3-day sample. The food materials were secured in amounts sufficient for each entire ex-

perimental period and analyzed for nitrogen; the Kjeldahl method was used for all determinations. In calculating digestibility, no correction was made for metabolic nitrogen; that is, apparent digestibility has been calculated in this experiment. Throughout the experiment the subjects were maintained on a positive nitrogen balance. The feces after the feeding of cabbage fiber were exceedingly bulky. The very liberal amount of meat fed represented a new experience for these subjects, and each of them gained 4 to 5 kilos weight during the 3 weeks under experiments.

TABLE 4

Food intake of human subjects on high roughage diet (totals for 3-day periods)

	AVERAGE NITROGEN CONTENT, PER CENT	PERIOD 1, GM.	PERIOD 2, GM.	PERIOD 3, GM.
Meat (lean)	3.26	1313	1346	1355
Rice[1]	1.53	450	630	630
Lotus root flour	0.50	450	390	390
Sugar	—	135	135	135
Salt vegetable	0.73	150	150	150
Cabbage fiber (dry)	1.40	—	150	—
Cabbage fiber (moist)	1.33[1]	—	—	60[1]
Total nitrogen (gm.)		57.4	57.0	55.0
Total calories (calculated)		8505	8694	8729
Total crude fiber (gm.)		4	80	35
		Per cent	*Per cent*	*Per cent*
Fraction of total nitrogen furnished by:				
meat		82.3	73.9	75.3
rice		11.8	17.0	17.6

[1] Weights recorded on dry basis.

The results are shown in table 5. The cabbage fiber as prepared was found to take up water rapidly and in the second period the subjects drank a large amount of water with their meals. There was a very pronounced sensation of distention, though in no case was there one of real discomfort. This method of ingesting dry cabbage substance followed by water is not the normal method of consuming dietary roughage, but it offered a convenient means of feeding a large bulk of material. In the third period the cabbage fiber was eaten

in moist form, as nearly as possible in the condition in which it occurs in the normal Chinese diet. The actual amount of crude fiber consumed during this last period, as indicated in the table, was somewhat less.

DISCUSSION

The results recorded above indicate that with rats the addition of filter paper, clay, and rice chaff, certainly up to 50 per cent of the diet, does not result in a significant reduction in digestibility of the protein fraction of the food. For levels above 50 per cent there is some indication of slightly lowered digestibility. It must be pointed out, however, that these

TABLE 5

Effect of cabbage fiber on the protein digestibility of a meat-rice diet in man (for 3-day periods)

SUBJECT	PERIOD	NITROGEN INTAKE, GM.	FECAL NITROGEN, GM.	DIGESTIBILITY[1]
A	1 (without fiber)	57.4	5.1	92.3
	2 (dry cabbage fiber)	57.0	5.7	90.0
	3 (moist cabbage fiber)	55.0	3.8	93.1
B	1 (without fiber)	57.4	4.0	94.2
	2 (dry cabbage fiber)	57.0	7.8	86.3
	3 (moist cabbage fiber)	55.0	3.8	93.1

[1] Metabolic nitrogen has been neglected in this calculation.

higher levels involve a lowered protein consumption, and probably also a greater amount of metabolic nitrogen, both of which tend toward inaccuracy in the calculation of digestibility.

When agar agar was incorporated into the diets with rats a speedier evacuation of food material from the alimentary tract was produced and this was invariably accompanied by lowered digestibility figures. Rice chaff did not produce laxative effects. This is in interesting contrast to bran which is known to possess outstanding laxative properties.

In the experiment using human subjects (tables 4 and 5), it was found that cabbage fiber added to the diet to an amount

of 12 gm. of crude fiber per day did not lower the degree of digestibility (period 3). When the level of fiber intake was placed at twice that amount (period 2), however, there was a slightly lowered value for the digestibility and also evidence of laxative action. Cabbage fiber is not so laxative as other roughage substances that often enter into the diet.

In all the experiments we have used as the sources of protein, materials whose protein digestibility is high, and we have aimed to produce just as large bulk effects as possible. In one of the experiments with human subjects the diet contained almost 30 gm. of crude fiber per day. While the weight of water-free fiber may not be a measure of the roughage effect of fiber in food, it is evident that the effective bulk of the contents of the digestive tract in these experiments was exceedingly large. Experiments hitherto reported in the literature do not conceive of amounts of bulk and roughage in the amounts in which they occur in the vegetarian diets of the Orient. The experiments of Whitacre, Willard and Blunt ('29) for example were carried out on diets which contained, according to our calculation, about 5 or 6 gm. of crude fiber per day.

The addition of clay to the food intake is not limited to stress of famine, but in some districts represents a normal habit. Kaolin, of course, is in use clinically; it acts presumably as an adsorbent for pathogenic organisms in the intestinal tract. Clay eaters are to be found in many parts of China, and individuals who indulge in this habit report that clay has a smooth, pleasant taste and produces a feeling of well-being. Of the various roughage substances employed in our experiments with rats, the clay diets alone were consumed with great eagerness by the animals; they manifested this appetite even for mixtures containing 80 per cent of the clay. The only obstacle was the difficulty of securing enough genuine food nutrient at these high levels of clay intake.

A lowered digestibility of protein in diets high in certain roughage materials would appear to have three possible explanations: 1) a bulk effect, where the large amount of ma-

terial may interfere with the action of proteolytic enzymes; 2) an exceptionally large output of metabolic nitrogen, 3) an unusually rapid passage of material along the alimentary tract, resulting in incomplete absorption.

The experiments here reported, where inert substances such as clay and pure cellulose filter paper were added to the diet, indicate that there is no bulk effect leading to reduced digestibility. On the other hand, if metabolic nitrogen, which may perhaps be regarded as an index of the amount of digestive juices secreted, increases with a bulkier food intake, it would seem plausible to suggest that the alimentary tract automatically counteracts a threatened reduction in digestibility due to bulk by causing an increased flow of digestive juice. It should also be pointed out that the roughage material may actually augment the reactive surface in the alimentary tract and thus tend to promote rather than impede digestibility.

The metabolic nitrogen is a complicating factor in all digestibility experiments. Just how much of the fecal nitrogen represents undigested food substances and how much represents unabsorbed metabolic residues is difficult to determine. The results with inert materials like clay recorded in this report show at least that these substances do not react on the intestinal mucosa in such a way as to yield excessive amounts of metabolic nitrogen.

Laxative action may be said to be characterized by softer, more watery stools, and by a more rapid passage of food through the alimentary tract. It would appear that not all bulky foods are laxative in character. Others are laxative to varying degrees; and crude fiber itself from different sources apparently differs in laxative value. Ordinary cellular food fiber is a milder laxative than agar agar. Many of the cases of lowered digestibility with fiber-rich diets, bran bread, etc., reported in the literature may be best attributed to this type of action which shortens the time allowed for digestion and absorption. Our own experience, as well as that of many others, with metabolism studies on man indicates that the degree of protein digestibility varies considerably in mixed

diets. Ferrari ('32), for example, finds that the addition of meat increases the amount of bread utilized in the diet, and concludes that nitrogen digestibility is only reduced in those cases where the passage of food material through the intestinal tract is more rapid than usual. Baumann ('32), studying intestinal action on a diet of apples, a food material especially rich in fiber, also concludes that such substances act mainly to accelerate the passage of material through the intestines.

SUMMARY

Filter paper, China clay, and rice chaff have been fed to rats at different levels with a cooked rice diet. The results indicate that neither varying the total amount of food nor adding large amounts of these bulk materials produces any significant effect upon the degree of digestibility of the food protein. China clay was fed in levels up to 80 per cent of the total intake. It would appear that great bulk in the alimentary canal does not interfere with the action of proteolytic enzymes.

Agar agar caused a rapid passage of the food material through the alimentary tract and showed distinctly lowered values for protein digestibility.

An experiment with two human subjects in which cabbage fiber was fed with a meat-rice diet showed a slight tendency toward a lowered degree of protein digestibility only when the fiber was ingested in an abnormally large amount.

It is suggested that lowered values for nitrogen digestibility on a given diet result only when the food material passes through the alimentary tract with unusual rapidity.

LITERATURE CITED

BAUMANN, T. 1932 Das chemische Stuhlbild und die Darmfunktion der Säuglinge und Kleinkinder bei Apfelkost. Klin. Wochschr., Bd. 11, S. 1267.

BOAS FIXSEN, M. A., AND H. M. JACKSON 1932 The biological values of proteins. III. A further note on the method used to measure the nitrogenous exchange of rats. Biochem. J., vol. 26, p. 1919.

FERRARI, R. 1932 Die Ausnutzung gemischter Nahrung. Arch. ges. Physiol., Bd. 230, S. 215.

FREY, J. W., E. R. HARDING AND T. R. HELMBOLD 1928 Dietetic investigations of edible pure cellulose. Med. J. Rec., vol. 127, p. 585.

MITCHELL, H. H. 1924 A method of determining the biological value of protein. J. Biol. Chem., vol. 58, p. 873.

RUBNER, M. 1918 Die Verdaulichkeit der Vegetabilien. Arch. Anat., Physiol. Abt., Jahrg. 1918, S. 53.

WEINERT, H. 1931 Der Einfluss von Sand und Schmutz auf die Verdaulichkeit der Nahrstoffe und den Milchertrag. Zeit. Tierzücht. Züchtungsbiol. Tierernahr., Bd. 22, S. 76.

WHITACRE, J., A. WILLARD AND K. BLUNT 1929 Influence of fiber on nitrogen balance and on fat in the feces of human subjects. J. Nutrition, vol. 2, p. 187.

THE BIOLOGICAL AVAILABILITY OF SOYBEAN CARBOHYDRATE

WILLIAM H. ADOLPH AND HSUEH-CHUNG KAO
Department of Chemistry, Yenching University, Peiping, China

(Received for publication July 14, 1933)

While soybean protein has received much attention, the study of soybean carbohydrate has been largely neglected. Soybean contains about 40 per cent of protein and 30 per cent of carbohydrate, the latter being analyzed as 'carbohydrate by difference.' This carbohydrate fraction contains little if any starch, but consists mainly of pentosans, galactans, hemicelluloses, etc., which it has been vaguely supposed the animal body utilizes in part at least. Millions of people in the Orient consume the soybean as food, and are dependent upon it as a source of both protein and carbohydrate. There is a conspicuous lack of published data showing the degree to which soybean carbohydrate is utilized by the animal body.

Blondell (1880) reported the absence of starch in soybean. Meissl and Böcker (1883) however found that soybean does contain a small amount of starch, and Harz (1885) indicated that this varies with the degree of maturity of the plant. Levallois (1881) identified galactan in soybean; Schulze and Frankfurt (1894) proved the presence of cane sugar; and Borghesani ('07) found pentose and pentosan. The most complete analytical study of soybean carbohydrate of which we are aware is that of Street and Bailey ('15), who show that sugars, starch and dextrin constitute about one-fourth of the total carbohydrate and point out that these are presumably absorbed by the human body. The remaining three-fourths consists mainly of waxes, tannins, galactans, pentosans, and cellulose, which have been assumed to be non-available to the human body.

The time-honored method of determining digestibility by measuring food and fecal carbohydrate over a definite feeding period has been used by several investigators. Oshima ('05) reports two experiments which gave 78 per cent as the average digestibility of soybean carbohydrate. Bowers ('19), also using this method, concluded that soybean carbohydrate is 96 per cent digestible. Results by this method, it is recognized, are complicated by the influence of bacterial decomposition in the gut. Experiments with animals, carnivora as well as herbivora, have demonstrated that even filter paper disappears in the digestive tract (Rubner, '16; Thomas et al., '18), while Strauch ('13) reports that 6 to 16 per cent of cellulose was 'digested' by human subjects. This method obviously is not an accurate measure of biological value, and has not been used in this study.

For estimating the biological availability of carbohydrates it was proposed to employ the following methods: 1) determination of reducing sugars after hydrolysis with takadiastase in vitro; 2) the formation of glycogen and determination of blood-sugar level; 3) determination of extra glucose in phlorhizinized animals; 4) determination of the respiratory quotient. The last three in vivo methods were carried out using albino rats. Methods for determining the biological availability of carbohydrates have been reviewed by Morgan, Strauch, and Blue ('30) and also by McCance and Lawrence ('29).

EXPERIMENTAL

Materials

1. Soybean. A quantity of yellow soybean (Peking variety), secured from a local shop, was ground to pass through a 40-mesh sieve. This material on proximate analysis showed: moisture, 6.57 per cent; ash, 4.18 per cent; protein, 39.15 per cent; fat, 21.17 per cent; carbohydrate including fiber (by difference), 28.93 per cent.

2. Fat-free soybean. The meal as prepared above was defatted in a Soxhlet apparatus and air dried.

3. Fat-free soybean curd. Soybean curd (soybean cheese), which is essentially soybean protein (glycinin), obtained from a local shop was cut into thin sheets and dried at room temperature for 3 to 4 days. It was then washed with ether to remove most of the oil, dried again, ground, and finally extracted in a Soxhlet apparatus to remove the last traces of fat. This material was used as a source of protein for the control animals. Its proximate analysis gave: protein 77.80 per cent, ash 5.26 per cent, moisture 9.26 per cent, carbohydrate (by difference) 7.68 per cent.

4. Filter paper flour. Ordinary filter paper was cut into strips, digested in boiling normal hydrochloric acid for 1½ hours, and set aside until a fine suspension of cellulose fiber was obtained. It was then washed until free from acid, dried on the water bath and ground to a fine powder. This material was used as the carbohydrate in the negative control diet.

Experiments and results

Experiment 1. Determination of total reducing sugars on hydrolysis with taka-diastase. The in vitro method used by Olmsted ('20) in investigating the carbohydrates of food materials was followed. Duplicate samples of 5 to 10 gm. of the fat-free soybean meal were treated with boiling water, and then with taka-diastase at 37° for 17 hours, closely adhering to the details of the method cited. The total reducing sugar was determined in an aliquot after hydrolysis with hydrochloric acid by the method of Folin-Wu. Control determinations were made on the reagents. Duplicate determinations by this method showed that carbohydrate, corresponding to 27.1 per cent of the total carbohydrate in the whole soybean is digested.

Experiment 2. Glycogen formation and blood sugar level. The general method of Cori and Cori ('26) was followed in these determinations. Rats weighing 150 to 300 gm. were used, divided into groups as shown in table 2, litter mates being distributed among the groups. They were fed the diets shown in table 1. These diets were so arranged that the

carbohydrate should represent the varying factor. Soybean curd defatted as described above proved to be a practical source of soybean protein; it contained a small amount of carbohydrate which was allowed for in calculating the equivalents of the diets in table 1; it amounted to but 3 per cent of the total carbohydrate present.

The rats raised on the stock diet were fasted for 24 hours and divided into groups; each group was then fed the pre-

TABLE 1

Composition of diets used with rats in experiments 2, 3, 4

	DIET 1 POSITIVE CONTROL	DIET 2 NEGATIVE CONTROL	DIET 3 EXPERIMENTAL
	Per cent	*Per cent*	*Per cent*
Soybean curd (fat-free)	45.0	45.0	—
Soybean (fat-free)	—	—	70.0
Starch	23.0	—	—
Filter paper	—	23.0	—
Soybean oil	30.0	30.0	30.0
Salt mixture (Osborne and Mendel)	2.0	2.0	—
Equivalent to			
Protein	35.0	35.0	34.7
Carbohydrate	26.5	26.5	25.8
Fat	30.0	30.0	30.0
Ash	4.3	4.3	3.7
Moisture	4.2	4.2	5.8

scribed diet for 1 week to render the animals accustomed to the diet. Water was supplied ad libitum throughout all phases of the experiments. During this preliminary period of 1 week, the food intake was measured daily and the feces also preserved for subsequent examination. Following this, the rats were again fasted for 24 hours, and fed the prescribed diet for the next 24 hours. At the end of this feeding period the animals were killed by a blow on the head and decapitated. One-tenth cubic centimeter of blood was imme-

diately taken for blood sugar determination, using the Byrd micro technic ('24). The liver was at once removed, and liver glycogen was determined according to the Sahyun modification ('31) of the Pflüger method. In several cases, the body of the rat after removal of the intestinal tract was also immediately cut into pieces for determination of body glycogen by the technic of Ågren, Wilander and Jorpes ('31).

The results of this experiment are shown in table 2. This table also includes for comparison data obtained for three rats which had been fasted for 48 hours. The average figures for per cent of liver glycogen as well as those for blood sugar indicate that in nutritive value, soybean carbohydrate is to be ranked about midway between starch and cellulose.

The feces of fourteen of these rats on the three diets of this experiment were collected during the 7-day preliminary feeding period, air dried, and weighed. The animals averaged 200 gm. in weight and consumed an average of 10 gm. per day of the ration. The following figures represent the average weight of dried feces per 10 gm. of food intake.

Diet	*gm.*
1 (starch)	0.78
2 (cellulose)	3.04
3 (soybean carbohydrate)	1.49

It is evident that soybean carbohydrate cannot be regarded as an extremely high-residue food material, but falls between starch and cellulose.

Experiment 3. Urinary sugar with phlorhizinized albino rats. Olmsted ('20) fed phlorhizinized dogs a definite amount of vegetable food material and by determining the extra sugar excreted in the urine estimated the amount of carbohydrate utilized. In this experiment we have employed the diets shown in table 1. Both male and female rats were employed. They weighed from 200 to 350 gm. and had been fed on stock diet no. 5 consisting of kaoliang, millet, and soybean. During the period of phlorhizinization, they were fasted, and were kept in metabolism cages of the type devised by Levine and Smith ('25), which permitted complete collection of the urine

TABLE 2

Liver glycogen, body glycogen, and blood sugar in rats fed soybean carbohydrate

RAT NO.	DIET	BODY WT.	FOOD INTAKE	LIVER WT.	LIVER GLYCOGEN		BODY GLYCOGEN		BLOOD SUGAR PER 100 CC.
		Gm.	*Gm.*	*Gm.*	*Mg.*	*Per cent*	*Mg.*	*Per cent*	*Mg.*
1248		263	17	8.7	—	—	98	0.042	170
1249		263	22	8.7	—	—	92	0.042	182
1252	Diet 1	189	13	5.0	—	—	82	0.051	180
1171	(starch)	310	11	8.6	377	4.39	78	0.030	182
1300		130	7	4.5	50	1.15	43	0.040	162
1335	Positive	190	20	5.2	103	1.98	—		162
1337	control	196	15	5.2	125	2.40	—		180
1343		175	15	5.4	202	3.75	—		150
1164[1]		158	9	4.8	161	3.98	—		151
1180[1]		185	9	6.2	196	3.17	—		214
1190[1]		239	16	9.6	137	1.43	—		164
					Avg.	2.78		0.041	173
1250		173	13	5.8	—	—	60	0.042	200
1251		161	12	5.6	—	—	75	0.057	122
1234	Diet 2	169	11	4.7	—	—	106	0.075	151
1301	(cellulose)	126	10	4.3	46	1.08	50	0.049	150
1172	Negative	252	13	6.9	136	1.97	96	0.040	160
1344	control	146	14	4.6	24	0.52	—	——	148
1339		154	15	5.0	72	1.44	—	——	133
					Avg.	1.25		0.053	152
1163		213	11	6.5	147	2.23	—	——	142
1164	Diet 3	172	11	5.4	86	1.60	—	——	134
1181	(soybean	176	10	6.4	173	2.72	—	——	192
1193	carbohydrate)	236	12	7.1	93	1.31	—	——	150
1191		267	16	—	—	—	60	0.027	170
1236		146	9	6.1	—	—	27	0.042	162
1127	Experimental	252	13	6.0	234	3.73	63	0.030	141
1305	diet	135	12	5.5	69	1.26	87	0.080	176
1329		168	14	5.4	110	2.04	—	——	178
1338		183	15	5.9	113	1.90	—	——	144
					Avg.	2.10		0.045	159
1165		214	0	5.1	11	0.21			145
1179	Fasted	182	0	4.3	8	0.19			116
1192		222	0	5.4	12	0.22			100
					Avg.	0.21			120

[1] Rats 1164, 1180 and 1190 were fed diet 1 except that soybean curd (table 1) was replaced by casein.

free from feces. Water was available throughout the experiment.

Phlorhizin glycosuria was induced by a daily subcutaneous injection of 50 mg. of phlorhizin in 3 cc. of olive oil till the G/N ratio became practically constant. Urinary sugar was determined by the method of Folin and Berglund, and nitrogen by the micro method of Folin and Denis. Satisfactory G/N ratios were obtained within 2 or 3 days and the rats were then ready for the feeding tests. The G/N ratios varied between 2.55 and 3.83. Shorr, Loebel, and Richardson ('30) have shown that each rat gives its own characteristic G/N ratio. Five grams of the prescribed diet was then given to the rat until the entire food was eaten, which was usually accomplished within 12 hours. The extra sugar excreted in the urine, caused by the available carbohydrate in the diet, was calculated as the difference between total glucose and the glucose from protein. Almost half the rats when submitted to the feeding test refused to consume all the material placed in the food cup; a few developed diarrhoea and could not be used. The data reported herewith are for those animals only which had accustomed themselves to consuming the diet portion promptly when fed.

The complete data for only one of the rats are reproduced here (table 3); this table is typical of the protocols for the other animals tested. In no case was extra sugar produced on the negative control diet; it would seem that filter paper cellulose was not utilized by the rats. From the data of table 3, a utilization coefficient has been calculated, using the figures for extra glucose, by comparison with starch as 100, i.e., $3.53 \div 1018 \times 100 = 34.7$ per cent utilized.

For six animals, the average figures obtained for the per cent of soybean carbohydrate assimilated were 34.7, 25.8, 40.4, 45.0, 37.8, 47.2, giving a mean value of 38.5 per cent.

Experiment 4. Determination of the respiratory quotient. The method of the respiratory quotient has been used by Mattill ('23) in studying the effect of vitamin B on carbohydrate utilization. We employed stock rats weighing from

150 to 300 gm. They were fasted for 24 hours, and the basal respiratory quotient determination was then made in the same room in which the animals were usually kept. A simple closed-circuit apparatus patterned after that used by Wu and Chen ('29) was employed in which a desiccator formed the respiration chamber. The carbon dioxide was absorbed in

TABLE 3

Effect of soybean carbohydrate on urinary sugar of phlorhizinized rats

Rat 1087 ♀. Body weight: before experiment, 278 gm.; after experiment, 210 gm.

DAY	FOOD INGESTED	TOTAL N IN URINE	TOTAL GLUCOSE IN URINE	G/N	GLUCOSE FROM PROTEIN (N × 3.83)[1]	CALCULATED EXTRA GLUCOSE
		Mg.	*Mg.*			*Mg.*
1	0	212	806	3.79	——	——
2	0	230	923	4.00	——	——
3	0	240	902	3.76	——	——
4	5 gm. diet 1 (starch)	331	2272	6.86	1268	1004
5	0	202	788	3.90	774	14
					Total	1018
6	5 gm. diet 3 (soybean carbohydrate)	219	1078	4.92	839	239
7	0	248	1064	4.25	950	114
					Total	353
8	5 gm. diet 2 (cellulose)	165	620	3.76	632	0
9	0	360	1296	3.60	1379	0
10	0	158	616	3.90	——	0
					Total	0

[1] Average G/N value for fasting rat 1087 = 3.83.

80 cc. of $\frac{1}{4}$ normal barium hydroxide, the excess of barium hydroxide being titrated with $\frac{1}{2}$ normal hydrochloric acid. The usual temperature corrections were applied in measuring the volume of oxygen and calculating the ratio CO_2/O_2. A preliminary series of ten determinations was made with fasting animals to ascertain that the apparatus was free from leaks and that checked results were possible.

The rats used in these preliminary trials were returned to the stock diet for 1 or 2 days, and then fasted for 16 hours to remove the influence of previous food. The three experimental and control diets (table 1) were then given ad libitum for intervals of 12 hours with an interval of 16 hours' fasting between each successive diet; 6 to 10 gm. of the food were consumed in each period. At the end of each feeding period each rat was placed in the apparatus for 1 hour, and the respiratory quotient determined for four 15-minute periods. The measurement for the first 15-minute period was always discarded; the recorded figure represents the average of the other three.

TABLE 4

Respiratory quotients with rats fed soybean carbohydrate

RAT NO.	FASTING	DIET 3 (SOYBEAN CARBOHYDRATE)	DIET 1 (STARCH)	DIET 2 (CELLULOSE)
1238 ♀	0.80	0.91	0.98	0.77
1238 ♀	0.82	0.88	1.01	0.83
1312 ♂	0.77	0.83	0.85	0.81
1308 ♂	0.75	0.92	0.98	0.79
Average	0.79	0.89	0.96	0.80

Results are shown in table 4. The respiratory quotient for diet 3 (soybean carbohydrate) lies between the figures obtained for diets 1 and 2, representing a biological value midway between starch and cellulose.

DISCUSSION

The four methods employed for the estimation of the biological availability of soybean carbohydrate give values varying between 27 and about 50 per cent for the fraction of total carbohydrate utilized by the animal body. Of these methods the in vitro method using taka-diastase gives the lowest value, possibly due to the fact that hemicelluloses which have nutritive value may escape hydrolysis. This method, however, still remains the most convenient rapid device for determining available carbohydrate content in food materials.

Experiments involving the determination of liver glycogen do not always lend themselves to a clear-cut interpretation of results. Fat has been shown by Gregg ('31) to yield a certain amount of glycogen; the same is true of protein in the diet. Moreover, it has been shown by Wilson and Lewis ('30) that different proteins may yield different amounts of glycogen. The arbitrary use of casein by some investigators in the control diet in glycogen experiments is therefore not above question. We have endeavored to eliminate these variables by using the same protein and the same fat in equal amounts in all the animal experiments, and in addition, both positive and negative controls were employed. It is impossible of course from the data of the glycogen experiment to assert that none of the cellulose in the negative control ration (diet 2) is converted into glycogen. Fürth and Engel ('31) report results which they interpret to indicate that cellulose forms liver glycogen. The other experiments here reported tend, however, to justify the assumption that the filter paper cellulose employed was not utilized by rats. It is of note that fasting animals give glycogen results differing widely from the animals fed a cellulose-protein-fat diet. Fasting animals cannot always serve as suitable controls. The data of table 2 also indicate that the change in the percentage figure for body glycogen is not so marked as that for liver glycogen. The experiment involving liver glycogen estimation indicated that approximately half of the soybean carbohydrate was utilized. That this method appears to yield a higher value than the taka-diastase method for the amount of soybean carbohydrate utilized is not surprising, since pentoses and hemicelluloses may also yield certain amounts of liver glycogen.

Experiments with phlorhizinized rats gave a utilization coefficient of 38 per cent, which also is somewhat higher than the figure obtained by in vitro digestion with taka-diastase. This is in agreement with the observation of Olmsted ('20), who also found higher values with phlorhizin experiments. We recovered as urinary sugar an average of 75 per cent of the starch fed to the control rats. Our results indicate that

phlorhizinized rats may be used for assaying the food value of carbohydrates.

The method of the respiratory quotient furnished an interesting check on the determination of carbohydrate utilized, but data from this type of experiment must be interpreted with caution, and this method does not commend itself as a routine method for the assay of carbohydrate in foods. The result obtained approximates that estimated from the liver glycogen method.

We have used the data of these experiments as a basis for approximating a utilization coefficient for soybean carbohydrate. The figure from taka-diastase digestion we believe to be low, while the experiments based upon the formation of liver glycogen and upon the method of the respiratory quotient involve complex diet factors. A consideration of the data from all four methods employed suggests that of the soybean fraction usually reported as 'carbohydrate by difference' about 40 per cent is available to the animal body. We are inclined to believe that hemicelluloses ordinarily regarded as non-utilizable are, in the case of the soybean, in part available.

SUMMARY

An experimental diet containing soybean carbohydrate, with positive and negative controls, was used with rats to measure the formation of liver glycogen, extra sugar in phlorhizinized animals, and the respiratory quotient. In vitro estimation of digestibility as well as these in vivo experiments indicate that about 40 per cent of the soybean carbohydrate is utilized by the animal body.

LITERATURE CITED

ÅGREN, G., O. WILANDER AND E. JORPES 1931 Cyclic changes in the glycogen content of the liver and the muscles of rats and mice. Biochem. J., vol. 25, p. 777.

BLONDELL, R. 1888 Observations sur la structure des graines de Soja hispida. J. de pharm. et de chim., T. 18, p. 537.

BORGHESANI, G. 1907 Sui pentosani della Soia hispida. Staz. Sper. Agrar. Ital., vol. 40, p. 118.

BOWERS, W. G. 1919 Some studies on the nutritive value of the soy bean in the human diet. No. Dak. Agr. Exp. Sta., Spec. Bull., vol. 5, p. 278.

BYRD, T. L. 1924 A blood diluting pipet for micro-Folin-Wu method of blood sugar estimation. J. Am. Med. Assn., vol. 83, p. 508.

CORI, C. F., AND G. T. CORI 1926 The fate of sugar in the animal body. II. The relation between sugar oxidation and glycogen formation in normal and insulinized rats during the absorption of glucose. J. Biol. Chem., vol. 70, p. 557.

FÜRTH, O., AND P. ENGEL 1931 Ueber die Verwertung der Pentosane im tierischen Organismus. Biochem. Zeitschr., Bd. 237, S. 159.

GREGG, D. E. 1931 Glycogen formation and respiratory quotients in rats fed exclusively on fat. J. Nutrition, vol. 4, p. 385.

HARZ, C. O. 1885 Ueber den Stärkegehalt der Sojabohne. Z. Allg. Oesterr. Apoth. Ver., Bd. 23, S. 40.

LEVALLOIS, A. 1881 Sur la matière contenue dans la graine du Soja hispida. Compt. rend., T. 93, p. 281.

LEVINE, H., AND A. H. SMITH 1925 A cage device for the study of ketosis and nitrogen metabolism in small animals. J. Lab. and Clin. Med., vol. 11, p. 168.

MATTILL, H. A. 1923 The utilization of carbohydrate by rats deprived of vitamin B. J. Biol. Chem., vol. 55, p. 717.

MCCANCE, R. A., AND R. D. LAWRENCE 1929 The carbohydrate content of foods. Med. Res. Coun. Spec. Rept. Ser., no. 135.

MEISSL, E., AND F. BÖCKER 1883 Ueber die Bestandtheile der Bohnen von Soja hispida. Monatshefte für Chem., Bd. 4, S. 349.

MORGAN, A. F., C. M. STRAUCH AND F. BLUME 1930 The nature and biological availability of almond carbohydrates. J. Biol. Chem., vol. 85, p. 385.

OLMSTED, W. H. 1920 Availability of carbohydrate in certain vegetables. J. Biol. Chem., vol. 41, p. 45.

OSHIMA, K. 1905 A digest of Japanese investigations on the nutrition of man. U. S. Dept. Off. Exp. Sta. Bull. No. 159.

RUBNER, M. 1916 Die Verdaulichkeit reiner Zellulose beim Hund. Arch. Anat. Physiol., physiol. Abt., Jahrg. 1916, S. 159.

SAHYUN, M. 1931 Determination of glycogen in tissues. J. Biol. Chem., vol. 93, p. 227.

SCHULZE, E., AND S. FRANKFURT 1894 Ueber die Verbreitung des Rohrzuckers in den Pflanzensamen. Ber. Chem. Ges., Bd. 27, S. 62.

SHORR, E., R. O. LOEBEL AND H. B. RICHARDSON 1930 Tissue metabolism. I. The nature of phlorhizin diabetes. J. Biol. Chem., vol. 86, p. 529.

STRAUCH, F. W. 1913 Fein zerteilte Pflanzennahrung in ihrer Bedeutung für den Stoffhaushalt. Z. Exp. Path. Therap., Bd. 14, S. 462.

STREET, J. P., AND E. M. BAILEY 1915 The carbohydrates and the enzymes of the soybean. J. Ind. Eng. Chem., vol. 7, p. 853.

THOMAS, K., H. PRINGSHEIM, W. FRITZE, R. KINDERMANN AND H. SCHOTTE 1918. Die Verdaulichkeit der Zellulose. Vergleichende Untersuchungen. Arch. Anat. Physiol., physiol. Abt., Jahrg. 1918, S. 25.

WILSON, R. H., AND H. B. LEWIS 1930 Comparative studies of the metabolism of amino acids. III. The formation of glycogen after oral administration of amino acids to white rats. J. Biol. Chem., vol. 85, p. 559.

WU, H., AND T. T. CHEN 1929 A method for the determination of respiratory exchange of small animals. Chinese J. Physiol., vol. 3, p. 307.

THE EFFECT OF THYROIDECTOMY AND THYROID FEEDING ON THE MILK SECRETION AND MILK FAT PRODUCTION OF COWS

W. R. GRAHAM, JR.
Department of Biochemistry, University of Toronto, and Department of Animal Husbandry, Ontario Agricultural College, Guelph

(Received for publication June 28, 1933)

It has been often suggested that the initiation and cessation of the lactation cycle are regulated by hormones. While investigations have been made using organ extracts it cannot be said that the control of milk secretion has been fully explained. Fellner ('31) concludes the action of the hormones of reproductive organs in the relation to milk secretion involves two factors: one stimulating growth and development of the gland during gestation, the other inhibiting the secretion of milk by the developing gland. The removal of the placenta at parturition supposedly removes this inhibition of lactation. The investigations of Turner and his co-workers ('30, '31) fully bear out the secretion of a growth promoting substance by the corpus luteum, but the initiation of secretion is ascribed to the appearance of a stimulating agent rather than to the removal of an inhibitor. This stimulating principle has been identified by Corner ('30), Turner and Gardner ('31), and Gueter and Stricher ('29) as a product of the anterior lobe of the pituitary. Its relationship with the corpus luteum secretion has been demonstrated by Nelson and Pfiffner ('31).

While the general lactation cycle may be controlled by these hormones there is a certain amount of more or less indefinite evidence indicating that metabolic rate may be an additional factor especially in the production of milk fat.

Relatively large day-to-day variations in milk fat percentage and in milk fat production are common knowledge to all those familiar with the records of dairy cattle. It is also well known that usually the percentage of fat is lower at the morning milking than at the evening milking. McEwan and Graham ('33), Campbell ('31, '32) demonstrate this clearly. The latter author shows that there is a distinct decline in the absolute amount of fat found in morning milkings and attributes this to some factor at work at night. Bartlett ('29) has pointed out that the milk fat percentage may be affected by the stage of lactation of the animal. It tends to rise near the end of the period. Clothier ('22), Ragsdale and Turner ('22), Buchanan and Lowman ('29), and McEwen and Graham ('33) conclude that the percentage of fat in milk is highest during the winter months and lowest during the early summer. That environmental temperature is a factor influencing the milk fat percentage was shown by Hays ('26) and confirmed by Weaver and Mathews ('28). These authors show that the per cent of fat in milk is inversely proportional to the temperature. Gowan ('24) has shown that the per cent of fat in the milk of Holstein cows decreases with age after the first lactation cycle of the cow.

While it is dangerous to draw conclusions regarding the production of milk fat from milk fat percentage data, as will be pointed out below, the work cited regarding the factors influencing the daily variations in milk fat may be shown to be compatible with a theory which postulates that a relationship between milk fat production and metabolic rate exists. There appears to be an increase in milk fat under conditions in which increased metabolism may be predicted from the experimental procedure, and vice versa. The data gathered by Campbell give support to such a theory. Much of the remaining work is, however, complicated by changes in the values of milk secretion and fat percentage as neither are constant nor are the variations in the same direction.

EXPERIMENTAL

No calorimeter was available for the determination of the relationship between metabolic rate and production of milk and milk fat. Thyroidectomy and thyroid feeding were therefore used to alter the metabolic rate of animals in the experiment to be described below.

Cows from the Ontario Agricultural College herd were used. These cows were milked twice daily, at 6 A.M. and 5 P.M., throughout the experiment. The milk was weighed to the nearest 0.5 pound and sampled as described in the official method for fat determination. Analyses for fat were carried out by the Babcock method.

During the experimental period no changes were made from the ration ordinarily fed to milking cows, except when thyroid was included in the diet and when supplements were made to the food of cow no. 45, as outlined below. The animals were confined to the stable at all times.

The thyroid fed was prepared from frozen thyroid glands obtained weekly from the abattoir. This material was minced in a meat chopper and dried in acetone and ether. Final traces of ether were removed from the filter press cake with a draft of warm air, and the dried material was ground in a Wiley mill. Since fresh lots of glands were dried weekly and the amount of moisture in the final product was not constant, feeding was done on the basis of the original weight of the glands before drying.

The effects of thyroidectomy on milk and milk fat secretion were studied on three cows. The thyroid glands were removed from cow no. 45 at the end of the first week of the experiment. Similar operations, but without removing the thyroids, were performed on cows nos. 21 and 33 for control purposes. Two weeks after the control operation the thyroids were removed from cow no. 21. Thus the results of thyroidectomy may be compared not only with the normal but with data gathered from animals which underwent a precisely similar operation, except that the thyroids were left intact.

When effects of the removal of the thyroid glands had been effectively demonstrated an attempt was made to change the milk and fat secretion by feeding desiccated thyroid glands, prepared as outlined above. Various levels of this material were fed and the milk and fat production during these periods were recorded. An attempt was also made to increase the fat production of no. 45 by adding to the diet substances which might be possible precursors of milk fat. After a long normal period on two cows, nos. 5 and 6, shown to be in falling lactation, the addition of small amounts of thyroid to their diet showed clear-cut results.

TABLE 1

Cow no. 33

WEEK	MEAN DAILY MILK YIELD (LBS.)	TOTAL WEEKLY FAT YIELD (LBS.)	PER CENT OF FAT IN MILK	REMARKS
1	26	7.76	4.2	Normal
2	15	4.64	4.3	Operation
3	14	4.19	4.4	
4	10	3.45	4.9	
5	9	2.54	4.1	
6	11	3.20	4.2	
7	9	2.64	4.2	
8	8	2.18	4.0	
9	7	2.30	4.7	

The milk secretion is presented as the mean daily yield of milk during the week. The twice daily analyses have been reduced in most cases to their mean values for the week. The fat production is represented as the total amount of fat produced during the week. The fat percentage is the percentage that the total fat production for the week is of the total milk secretion for the same period.

RESULTS AND DISCUSSIONS

Milk secretion. Table 1 is a condensation of the data gathered on the milk secretion from Holstein cow no. 33. A normal period of 1 week is presented, followed by a control operation without thyroidectomy.

Table 2 presents, in condensed form, the data gathered for the milk secretion of Holstein cow no. 21 during the whole experiment. A normal period of 2 weeks is shown. The control operation was then performed and after another 2-week interval the thyroids were removed.

Table 3 similarly presents results with the Ayrshire cow no. 45. A thyroidectomy operation was performed at the end of the first week of the experiment.

From the daily results of the control operations shown in tables 8 and 9 it appears that the curve of milk secretion regains its normal slope within a week's time. The small secondary fall in milk secretion, shown in table 8, after apparent recovery, is possibly due to the fact that the incision became infected at this time and had to be drained. However, in any case recovery is apparently complete by the end of the third week after the control operation.

A comparison of the curve of milk secretion of cow no. 21 after the control operation and after thyroidectomy, shown in table 2, indicates little difference in the effect produced. This may be due to the fact that the second operation reduced the milk secretion to a very small amount. No further fall could take place without abolishing secretion entirely.

Examination of the data presented in table 3 shows that in the case of cow no. 45 there was a distinct fall in milk production for 5 weeks following thyroidectomy, followed by a flattening out of the downward slope of the curve of secretion. From tables 8 and 9 it would appear that this prolonged fall in milk secretion might possibly be attributed to thyroidectomy, and not to the simple effect of operation. However, even if thyroidectomy per se produces a diminution in the milk secretion of cows, as indicated in table 3, the results shown above are difficult to interpret because of the somewhat similar effect after the control operations.

The effect of thyroid feeding

Since the decreases in milk secretion after operations could not be definitely associated with removal of the thyroid

TABLE 2

Cow no. 21

WEEK	MEAN DAILY MILK YIELD (LBS.)	TOTAL WEEKLY FAT YIELD (LBS.)	PER CENT OF FAT IN MILK	REMARKS
1	19.0	5.55	4.2	
2	17.0	4.90	4.1	
3	12.4	3.64	4.2	Operation
4	7.7	2.17	3.9	
5	4.6	1.21	3.7	Thyroidectomy
6	3.1	0.99	4.5	
7	4.1	1.07	3.8	
8	3.8	0.95	3.5	
9	3.8	0.89	3.3	
10	3.7	0.89	3.4	
11	5.0	1.02	2.9	
12	39.8	12.53	4.5	1.3 lbs. thyroid fed daily: calved
13	45.6	10.67	3.3	No thyroid
14	46.9	13.92	4.2	1.9 lbs. thyroid fed daily
15	50.3	13.83	3.9	1.3 lbs. thyroid fed daily
16	51.4	12.99	3.6	0.66 lb. thyroid fed daily
17	50.3	14.67	4.2	0.66 lb. thyroid fed daily
18	53.3	12.68	3.4	0.66 lb. thyroid fed daily
19	54.6	12.70	3.4	0.66 lb. thyroid fed daily
20	54.6	13.09	3.4	0.66 lb. thyroid fed daily
21	44.4	10.38	3.3	0.66 lb. thyroid fed daily (no thyroid 2 days)
22	49.9	10.39	3.4	0.66 lb. thyroid fed daily
23	46.2	11.76	3.7	0.75 lb. thyroid fed daily
24	43.2	12.10	3.6	0.75 lb. thyroid fed daily
25	46.0	10.89	3.5	0.75 lb. thyroid fed daily
26	45.2	11.38	3.5	0.75 lb. thyroid fed daily
27	43.3	11.16	3.8	0.75 lb. thyroid fed daily
28	41.9	11.64	3.3	0.75 lb. thyroid fed daily
29	39.5	9.72	3.3	0.75 lb. thyroid fed daily
30	40.5	9.86	3.5	0.75 lb. thyroid fed daily
31	42.0	10.95	3.7	1.50 lbs. thyroid fed daily
32	39.0	10.19	3.7	0.50 lb. thyroid fed daily
33	39.3	9.65	3.5	0.50 lb. thyroid fed daily
34	39.4	9.06	3.4	0.50 lb. thyroid fed daily
35	33.1	7.81	3.4	No thyroid
36	25.8	5.80	3.2	No thyroid
37	25.6	5.28	2.9	No thyroid
38	25.5	5.18	2.9	No thyroid
39	25.5	5.52	3.1	No thyroid
40	29.0	6.20	3.0	0.50 lb. thyroid fed daily
41	30.3	7.78	3.7	0.50 lb. thyroid fed daily

glands, an attempt was made to study the effects of small amounts of desiccated thyroid glands in the diet of these

TABLE 3
Cow no. 45

WEEK	MEAN DAILY MILK YIELD (LBS.)	TOTAL WEEKLY FAT YIELD (LBS.)	PER CENT OF FAT IN MILK	REMARKS
1	35.0	12.44	5.1	Normal
2	34.5	11.33	4.7	Thyroidectomy
3	31.3	8.56	3.9	
4	28.0	7.57	3.9	
5	25.8	6.95	3.9	
6	23.8	6.71	4.0	
7	24.4	6.82	4.0	
8	21.8	6.66	4.4	
9	23.0	7.18	4.5	1.3 lbs. thyroid fed daily
10	28.3	9.35	4.7	1.3 lbs. thyroid fed daily
11	30.2	8.60	4.1	1.3 lbs. thyroid fed daily
12	31.5	8.72	3.9	1.3 lbs. thyroid fed daily
13	29.2	7.76	3.8	1.3 lbs. thyroid fed daily
14	27.7	7.55	3.9	1.3 lbs. thyroid fed daily
15	27.6	7.31	3.8	2.6 lbs. thyroid fed daily
16	26.4	7.24	3.9	2.6 lbs. thyroid fed daily
17	21.8	6.49	4.2	2.6 lbs. thyroid fed daily
18	22.3	5.96	3.8	2.6 lbs. thyroid fed daily
19	21.0	5.67	3.8	+ 2 lbs. sugar added daily
20	19.5	5.83	4.5	+ 2 lbs. sugar added daily
21	14.7	4.59	4.5	No sugar
22	15.5	3.85	3.5	+ 4 lbs. honey added daily
23	14.0	3.76	3.8	+ 1 lb. butterfat added daily
24	13.4	3.68	3.9	+ 1 lb. butterfat added daily
25	11.3	3.09	3.9	+ honey + fat + 1 lb. beet molasses
26	10.6	2.79	3.7	+ honey + fat + beet molasses
27	10.3	2.56	3.6	+ honey + fat + beet molasses
28	8.7	2.32	3.8	+ honey + fat + beet molasses
29	7.9	1.88	3.4	Thyroid alone
30	6.8	1.74	3.6	+ 1 lb. dried egg yolk
31	7.0	1.78	3.6	+ 2 lbs. dried egg yolk
32	4.9	1.46	4.2	No thyroid
33	1.8	0.64	5.1	No thyroid

animals. Thyroid glands, dried in the manner described above, were added to the diets of the thyroidectomized animals 21 and 45. The resulting effects are shown in tables 2 and 3.

Cow no. 21 received thyroid in the diet during the twelfth to the thirty-second weeks of the experiment. It is unfortunate that the initial effect of thyroid feeding on milk secretion is obscured by the effects of parturition. Thyroid feeding was discontinued during the thirty-fifth to the thirty-ninth weeks of the experiment. A distinct decline in milk secretion from 39.4 pounds on the preceding week to 25.8 pounds on the thirty-sixth week occurred. Resumption of thyroid feeding was followed by a rise in milk secretion. The results obtained when this cow was deprived of thyroid during the thirty-fifth to the thirty-ninth weeks simulate the effects of thyroidectomy and suggest, therefore, that the lessened milk production after thyroidectomy is indeed due to depriving the animal of its normal thyroid secretion.

The thyroidectomized cow, no. 45, was fed thyroid during the eighth to the thirty-first weeks of the experiment. As shown in table 3, this was followed by a rapid rise in milk secretion almost to the preoperative level.

These results indicate that thyroid feeding definitely increases the daily milk yield of thyroidectomized cows. If a similar effect could be shown with normal animals it would provide additional evidence that the thyroid is a factor in milk secretion. Accordingly, two Holstein cows, nos. 5 and 6, were chosen for experimental feeding. These animals were observed during a normal period of 11 weeks and were shown to be definitely in a state of falling lactation. At the end of this time an amount of thyroid equal to half a pound of the original glandular material was added to the daily ration of each animal. The consequent rise in milk secretion is shown in tables 4 and 5. Following the 4-week period thyroid feeding was discontinued and the secretion of milk rapidly fell to the levels which would have been reached in the absence of added thyroid. These results, together with those obtained with thyroidectomized animals, indicate that the thyroid gland has a definite influence on the amount of milk secreted by the cow.

THE PRODUCTION OF MILK FAT

The production of milk fat may be considered in two ways, namely, from the percentage of fat in the milk secreted and the total amount of fat produced. It is obvious that variations in the total amount of fat produced represent true variations. On the other hand, variations in the percentage of fat in the milk may be due either to variations in milk secretion or in fat production or to variation in both.

The effect of thyroidectomy and thyroid feeding will be considered in the sequence followed in discussing milk secretion.

The effect produced by a control operation on the production of milk fat is similar to that produced on milk secretion (tables 1, 2, 8, and 9). A sharp drop in production is apparent. The decrease continues for about 4 weeks, when equilibrium is reached (table 8). It is important to note, however, that after the control operation the per cent of fat in the milk of these animals is not below the limits of normal variation. Fat production and milk secretion fall at essentially the same rate.

The demonstration of the effect of thyroidectomy on the production of milk fat in cow no. 21, apart from any effect which might be due to operative disturbance without thyroid removal, is complicated by the fact that milk secretion fell almost to a minimum on the first few days after the operation. In order therefore to reproduce the effect of thyroidectomy, the dried thyroid was removed from the diet of this animal on the thirty-fifth week of the experiment. This procedure removes any complications which might be caused by an operation. The data in table 2 show that on removal of the thyroid from the diet of this animal, fat production dropped from 9.06 pounds on the thirty-fourth week to 5.80 pounds on the thirty-sixth week, or a decline in production in 2 weeks' time of 3.26 pounds of fat, or 36 per cent of the former level. The per cent of fat in the milk over the same period declined from 3.4 per cent to 2.9 per cent. A similar decline was observed after thyroidectomy operation. It ap-

pears that removal of the thyroid glands from the lactating cow causes a sharp decline in the total yield of milk fat. The diminution in the production of milk fat is more rapid than that of milk secretion. It soon reaches and is maintained approximately constant at a new and lower level.

The effect of thyroid feeding

The milk fat production of thyroidectomized cows nos. 45 and 21 fell to a new level following thyroidectomy. After this level was well established for cow no. 45 an amount of thyroid equal to 1.3 pounds of the original glands was added to the daily diet on the ninth week of the experiment. Table 3 shows that the production rose from 6.66 pounds per week on the eighth week to 9.35 pounds on the tenth week, or that there was a rise of 2.69 pounds, equal to approximately a 40 per cent increase, above that produced in the absence of thyroid influence.

In the case of cow no. 21, 0.50 pound daily of thyroid gland was fed commencing on the fortieth week of the experiment. Table 2 shows that fat production rose from 5.52 pounds on the thirty-ninth week to 7.78 pounds on the forty-first week. This rise of 2.26 pounds is approximately equal to 40 per cent of the previous production in the absence of thyroid feeding.

The distinct increase in the fat yield accompanying thyroid feeding to thyroidectomized animals suggested its trial with normal cows. The two animals already referred to, nos. 5 and 6, were chosen for this work. Table 4 shows that the fat secretion of no. 5 rose, when the equivalent of 0.50 pound of thyroid gland was fed daily, from 5.22 pounds on the eleventh week to 7.60 pounds on the fourteenth week. Table 5 shows that the cow no. 6, similarly treated, increased its fat production from 6.77 pounds to 9.48 pounds over the same period.

Clear-cut increases in both milk and milk fat production have been shown to accompany additions of thyroid substance to the diet of both thyroidectomized and normal animals. Decreases were shown after thyroidectomy and after the removal of desiccated thyroid glands from the diet. These

results might be interpreted as an indication that the thyroid gland produces a hormone which controls milk secretion, or that one of the hormones which controls the lactation cycle produces its effect through the thyroid.

Examination of the data presented in tables 2 and 3 reveals that the thyroid probably contains no such principle.

TABLE 4

Cow no. 5

WEEK	MILK	FAT SECRETION	FAT PER CENT	REMARKS
1	34.1	6.68	3.3	Normal
2	34.7	7.63	3.1	
3	34.7	7.69	3.2	
4	33.3	7.35	3.1	
5	31.8	7.10	3.2	
6	30.0	6.49	3.1	
7	28.4	5.92	3.0	
8	27.1	6.16	3.3	
9	24.4	5.62	3.3	
10	21.8	4.95	3.2	
11	22.1	5.22	3.4	
12	24.6	6.09	3.5	0.5 lb. thyroid fed daily
13	27.5	7.18	3.7	0.5 lb. thyroid fed daily
14	28.2	7.60	3.8	0.5 lb. thyroid fed daily
15	27.1	7.08	3.7	0.5 lb. thyroid fed daily
16	23.0	6.25	3.9	No thyroid fed
17	19.6	4.94	3.6	No thyroid fed
18	18.5	5.09	3.9	No thyroid fed
19	17.5	5.31	4.3	No thyroid fed
20	14.8	4.35	4.2	No thyroid fed
21	11.2	3.58	4.6	No thyroid fed
22	9.4	2.97	4.4	No thyroid fed

After the removal of the glands there is a rapid initial drop in production of milk and milk fat, followed by a general smoothing out of the curves. Neither milk nor fat production is reduced to zero. It is also clear that the secretion of milk and milk fat continued to fall in each of the periods when thyroid was fed at constant levels in the later weeks of the experiment. These periods were not designed for the purpose of deciding whether the thyroid is the predominant factor controlling lactation and objection may be justifiably

raised to the use of all the data as negative evidence. However, that shown in table 2 for cow no. 21 from the twenty-third to the thirtieth weeks of the experiment may be used for this purpose without objection. During this period milk secretion declined from 46.2 to 40.5 pounds daily and fat production from 12.10 to 9.86 pounds weekly. This diminution is slow but definite and incompatible with a theory of primary control of the lactation cycle.

TABLE 5

Cow no. 6

WEEK	MILK	FAT SECRETION	FAT PER CENT	REMARKS
1	32.0	7.63	4.0	Normal
2	32.9	9.07	3.9	
3	33.9	9.20	3.9	
4	31.1	8.53	3.9	
5	29.1	9.78	3.8	
6	30.7	8.45	3.9	
7	27.1	7.61	4.0	
8	26.6	7.16	3.8	
9	27.5	7.49	4.1	
10	26.4	7.08	3.8	
11	24.4	6.77	4.0	
12	28.0	7.97	3.9	0.5 lb. thyroid fed daily
13	34.7	9.53	3.9	0.5 lb. thyroid fed daily
14	35.0	9.48	3.9	0.5 lb. thyroid fed daily
15	33.6	9.00	3.8	0.5 lb. thyroid fed daily
16	30.7	8.93	4.6	No thyroid fed
17	25.7	6.53	3.6	No thyroid fed
18	24.0	6.52	3.9	No thyroid fed
19	22.9	5.96	3.7	No thyroid fed
20	19.3	5.35	3.9	No thyroid fed
21	18.3	4.80	3.7	No thyroid fed
22	18.0	4.89	3.8	No thyroid fed

The results outlined above indicate that the removal of the thyroid glands from the animal, or the feeding of desiccated thyroid in the diet, simply alters the plane of secretion of milk and milk fat, and that the thyroid does not contain any principle which controls the lactation cycle.

Since the presence or absence of the thyroid gland shows a definite effect on the secretion of milk and milk fat, which

in the light of the data at hand cannot be attributed to the secretion of a substance directly controlling the activity of the mammary gland, the effect must be due to some secondary condition produced by thyroid action. The thyroid gland of course influences the rate of metabolism in the animal. In the absence of the thyroid the basal metabolic rate falls some 30 to 40 per cent, while additions of the gland to the diet are known to increase metabolic rate in direct proportion to the amount of the active principle, thyroxin, absorbed.

If the effects shown above are due to one factor, or the sum total of the factors of increased metabolism, it is reasonable to suppose that at any time in the lactation cycle of an animal, maximal secretion of milk or milk fat can be attained, other factors being equal, only when the rate of metabolism is optimal for milk production. Above this optimal rate there would be increased catabolism. Subsequent additions of more thyroid to the diet, under these conditions, should show no increase in the milk or milk fat production, but rather a decrease, and the digestion products, which with the cow are chiefly carbohydrate and protein in origin, would be consumed in the body and the possibilities of fat formation from carbohydrate would be remote. Thus the precursors of milk fat, which according to Meigs, Blatherwick and Cary ('19), Cockefair ('28), Peterson, Palmer and Eckles ('29), are fatty compounds, would diminish and the secretion of milk fat would consequently be lower than the secretion of the other constituents of milk. If the metabolic rate of the animal is below the optimum for maximal milk or milk fat secretion the factors which diminish the secretion of the mammary gland will be a reduction in metabolites, coupled with a lower blood supply to the gland due to decreased heart rate. Increasing the metabolic rate under such conditions should show an increase in milk and milk fat production which cannot, however, be maintained against the decline resulting from the progression of the lactation cycle.

While a theory as outlined above cannot be definitely proved without calorimetric data, an attempt has been made to show

the behavior of two thyroidectomized cows, one kept in a state of hyper-metabolism, the other in a state of hypo-metabolism. The amounts of thyroid fed were purely arbitrary and the state of metabolism was judged from such symptoms as respiration rate, heart rate, excitability, appetite, etc.

Cow no. 45 was fed a daily quantity of thyroid, from the ninth to the fourteenth weeks, equivalent to 1.3 pounds of the original glands. The downward slope of the curve of milk secretion, after its peak, due to the initial effect of thyroid, was reached and was slightly arrested by doubling the dose of thyroid on the fifteenth week. This was followed by a marked drop in secretion from 26 to 21 pounds daily on the seventeenth week. This animal was now definitely in a state of hyper-catabolism. An addition of sugar to the diet, at the rate of 2 pounds daily, apparently delayed a further diminution of milk and fat production. This took place on the twenty-first week when sugar was removed from the diet. On the twenty-second week this downward fall in milk secretion was changed to a slight rise by the addition of 4 pounds of honey to the diet. The addition of further supplements to the ration apparently forestalled any abrupt declines in the milk secretion until thyroid feeding was discontinued. The effect of doubling the thyroid on the milk fat was a further diminution of its production. The fat percentage in the milk showed a gradual decline to the end of the experimental thyroid feeding, coincident with falling milk secretion. This indicates that increasing the thyroid fed tended to diminish the fat secretion at a more rapid rate than milk secretion. This might be ascribed to an increase in metabolism above the optimum rate, as pointed out above.

With the object of changing the milk and milk fat production at will, by altering the amount of thyroid fed, an attempt was made to keep cow no. 21 at a rate of metabolism below the optimum for secretion. As shown in table 2 no distinct difference could be observed in milk secretion when the thyroid fed was raised from 0.66 pound daily on the twenty-third week of the experiment. However, at this time fat produc-

tion rose from 10.38 pounds on the twenty-second week to 12.10 pounds on the twenty-fourth week. On the thirty-first week of the experiment the thyroid fed was increased to the equivalent of 1.50 pounds daily. Milk secretion rose slightly, showing a gain for the week of 1.5 pounds daily, and fat production figures showed a gain of a little more than 1 pound over the previous week. From the thirty-second to the thirty-fourth weeks the equivalent of 0.50 pound of thyroid was fed. Milk secretion fell off slightly, while fat production diminished from 10.19 to 7.81 pounds. The fall in both milk and fat, when thyroid was removed from the diet and the rise when it was replaced, has been discussed previously.

While these experiments show little or no information as to the actual mechanism of the changes in milk and milk fat secretion, they do show that additions of thyroid to the diet of an animal already receiving a reasonable supply of this material, will cause a decrease in both milk and milk fat production. Since sharp declines were arrested by introducing additional food of high caloric value to the diet it appears that the factor involved is the metabolic rate. An animal fed smaller amounts of thyroid may, however, have its milk and milk fat production increased by changing the amounts of thyroid in the diet. These results are at least compatible with a theory that the thyroid exerts its influence through its control of metabolic rate.

The results obtained with cow no. 19, table 6, show that some other factor than thyroid secretion controls milk fat and milk production. This animal, with a previous history of abortion, was washed, fitted for the show ring and exhibited on the fifth and sixth days of the experiment. On the tenth day an amount of thyroid was fed equal to 0.50 pound of the original glands. The animal showed a distinct decline in both milk and milk fat before thyroid was fed, indicating that the abortion was probably brought about by the treatment received in preparation for exhibition. The addition of thyroid to the diet temporarily raised the plane of secretion, as it does with animals in falling lactation. This is shown by

an arrest in the curve of the fall of milk secretion and of fat production. However, the factors governing the secretion of milk, outside the thyroid, presently forced both milk and milk fat production to lower levels, showing that the effect produced by the thyroid was secondary to other factors governing milk secretion.

TABLE 6

Cow no. 19

DAYS	MILK (LBS.)	FAT (LBS.)	REMARKS
1	37.0	1.21	
2	35.5	1.27	
3	34.5	1.19	
4	31.0	1.05	
5	34.5	1.11	Fitted
6	33.0	1.18	Exhibited
7	31.0	1.17	
8	26.0	0.96	
9	26.0	0.85	
10	26.5	0.91	0.5 lb. thyroid fed daily
11	26.5	0.85	
12	26.0	0.76	
13	25.0	0.85	
14	25.0	0.89	
15	22.0	0.71	
16	21.5	0.74	
17	21.5	0.74	
18	20.5	0.74	
19	18.5	0.71	
20	18.5	0.80	
21	18.5	0.76	
22	18.5	0.77	Aborted
23	23.0	0.92	
24	26.5	0.92	
25	31.5	1.45	

It will be noted that the effect of thyroid has been considered only on animals past the peak of milk production in their normal lactation cycle. Further evidence of the secondary nature of the effect of thyroid is afforded by a consideration of its effects soon after parturition, when the factors controlling milk secretion are probably exerting their greatest effect.

Changes were made in the amounts of thyroid fed to cow no. 21, shown in table 2, directly after parturition, when milk and fat secretion were rising rapidly. The equivalent of 1.3 pounds of thyroid glands was being fed on the twelfth week, when this animal calved. Milk secretion rose at this time from 5 pounds to 39.8 pounds daily, and fat secretion from 1.02 pounds to 12.53 pounds for the week. On the thirteenth week no thyroid was fed. Milk secretion continued to rise, showing a gain of 5.8 pounds over the previous week. Fat secretion diminished 1.86 pounds for the week. Milk secretion continued to rise in the following weeks without regard to the amount of thyroid fed, as did fat production until a peak was reached. In the succeeding weeks, that is in the normal period of falling lactation, the effect of thyroid could again be demonstrated. It is of interest to note here that during the stage of increasing milk production in spite of the apparent ineffectiveness of thyroid on the milk secretion, some slight effects on fat secretion are apparent; also, that thyroid at this time was absolutely necessary to the well-being of the animal, which showed serious symptoms when the thyroid feeding was stopped and was only saved from death by restoring thyroid to its diet. Thyroid feeding was, therefore, necessary to maintain life in this thyroidectomized animal. During the thirteenth week the temperature of this cow rose to 107° and quickly fell to normal when thyroid feeding was resumed. The diminution in fat secretion at this time might have been caused by lack of thyroid but was more probably due to hyper-metabolism.

In order to confirm these results a normal cow, no. 14, was fed thyroid during the fourth and fifth weeks after calving. The data presented in table 7 show no distinct rise in milk secretion. The secretion of milk fat was actually diminished in this procedure. Magee ('24) has shown that the metabolic rate per unit of weight of goats rises to a peak at the fourth week after parturition, so that the addition of thyroid at this time probably caused hyper-metabolism, which probably caused the diminution in the milk fat. It is clearly shown,

therefore, from the behavior of these two animals that thyroid feeding during rising lactation directly succeeding parturition shows little or no influence on the milk secretion. Some effects, however, may be noted on fat secretion. These effects may be related to metabolic disturbance.

TABLE 7

Cow no. 14

Daily production of milk and milk fat

DAYS	MILK SECRETION (LBS.)	FAT PRODUCTION (LBS.)	REMARKS
1	24.0	0.66	Calved
2	46.5	1.30	
3	47.5	1.51	
4	51.0	1.85	
5	53.5	1.87	
6	58.0	2.13	
7	59.5	2.25	
8	60.5	1.94	
9	57.5	2.07	
10	59.5	2.13	
11	60.5	2.37	
12	62.5	2.20	
13	62.5	2.26	
14	62.5	1.97	
15	59.5	1.98	
16	60.5	2.35	
17	59.5	2.00	
18	64.0	2.42	
19	59.5	2.45	
20	60.0	1.98	0.5 lb. thyroid fed daily
21	57.0	1.70	
22	61.5	2.21	
23	63.0	1.70	
24	63.0	2.15	
25	63.0	1.89	
26	63.5	1.90	
27	63.5	1.71	
28	62.0	1.61	
29	63.0	2.18	
30	64.5	2.05	
31	64.0	1.82	
32	63.0	1.93	
33	64.0	1.89	

TABLE 8

Showing the effect of the control operation on the milk secretion and milk fat production of cow no. 33

DAYS	DAILY MILK YIELD (LBS.)	DAILY FAT YIELD (LBS.)	REMARKS
1	27.0	1.16	
2	25.0	1.11	
3	26.0	1.08	
4	26.0	1.05	
5	24.0	1.01	
6	25.0	1.00	
7	21.5	0.91	Operation
8	18.0	0.88	
9	19.0	0.75	
10	16.0	0.62	
11	16.0	0.67	
12	13.0	0.61	
13	13.0	0.62	
14	13.5	0.56	
15	13.0	0.57	
16	12.5	0.67	
17	14.5	0.60	
18	15.0	0.60	
19	14.0	0.58	
20	13.5	0.56	
21	13.5	0.61	
22	11.0	0.54	
23	11.0	0.57	
24	11.0	0.57	
25	10.5	0.55	
26	9.5	0.44	
27	8.5	0.37	
28	9.0	0.41	
29	9.0	0.49	
30	8.5	0.33	
31	8.5	0.35	
32	9.5	0.39	
33	8.0	0.35	
34	9.0	0.37	
35	9.5	0.36	

TABLE 9

Showing the effect of the control operation on the milk secretion and milk fat production of cow no. 21

DAYS	DAILY MILK YIELD (LBS.)	DAILY FAT YIELD (LBS.)	REMARKS
1	19.0	0.78	
2	19.5	0.83	
3	19.0	0.81	
4	19.0	0.79	
5	19.5	0.82	
6	19.0	0.78	
7	18.5	0.74	
8	18.0	0.75	
9	18.0	0.71	
10	17.5	0.71	
11	17.5	0.69	
12	16.5	0.67	
13	16.5	0.69	
14	16.0	0.68	
15	16.0	0.64	
16	15.0	0.63	Control operation
17	13.5	0.56	
18	11.5	0.53	
19	9.0	0.51	
20	8.5	0.39	
21	8.5	0.38	
22	8.5	0.36	
23	9.0	0.35	
24	9.0	0.35	
25	10.0	0.39	
26	9.5	0.35	
27	9.5	0.37	
28	8.0	0.31	
29	7.5	0.30	Thyroidectomy
30	3.0	0.25	
31	2.0	0.10	
32	1.0	0.09	
33	1.5	0.06	
34	1.	0.10	
35	3.	0.16	
36	3.5	0.13	
37	3.5	0.14	
38	3.5	0.14	
39	3.5	0.14	
40	3.5	0.14	

SUMMARY

The removal of desiccated thyroid glands from the diet of thyroidectomized cows causes a diminution in the amount of milk and milk fat produced. Thyroidectomy itself causes a marked lowering of milk fat secretion. The diminution in milk secretion following removal of the thyroid gland cannot be readily distinguished from that accompanying control operations.

The addition of small amounts of thyroid to the diet of either thyroidectomized or normal cows, when the curve of lactation is falling, causes a rapid rise in the milk and milk fat production to a higher level after which the gradual fall, which is normal during this period in the lactation cycle, continues. During the period of rising milk secretion, which persists some 4 to 6 weeks after parturition, thyroid feeding had no apparent effect on the amount of milk secreted. Slight differences in the amount of milk fat secreted at this time may be related to the amount of thyroid fed. Feeding excessive quantities of thyroid to a thyroidectomized animal caused a diminution in milk secretion and fat secretion. From reduced milk fat percentages at this time it is apparent that the reduction in fat secretion was greater than that in milk secretion.

Lactation was not reduced to zero when thyroid was removed from the diet of the animals in the experiment. The effect of deprivation of thyroid on the total quantity of milk and milk fat produced seemed to depend on the period in the lactation cycle when the thyroid was removed.

From the results of these experiments it appears that the effect produced by the thyroid glands on milk and milk fat secretion is secondary to those factors controlling the lactation cycle. The experimental results suggest a relationship between total metabolic rate and the secretion of milk and milk fat. Fat production in these experiments appears to be more affected by the presence or absence of thyroid in the diet than is milk secretion. These rapid changes in fat secretion are thus reflected in changes in the per cent of fat in the

milk. Hence it is possible that the characteristic fluctuations in fat production, and especially fat percentage, in the records of normal cows, may be due to slight changes in the total metabolic rate of the animals.

The author wishes to thank Dr. N. B. Taylor and Dr. R. A. McIntosh for performing the thyroidectomy operations; also Mr. H. Palmer who milked and cared for the cattle during the whole of the experiment.

LITERATURE CITED

BARTLETT, S. 1929 Normal day to day variability of yield of milk and fat of individual cows. J. Agr. Sci., vol. 19, p. 438.

BUCHANAN, J. H., AND O. E. LOWMAN 1929 Seasonal variations in the freezing point of milk. J. Dairy Sci., vol. 12, p. 484.

CAMPBELL, K. W. D. 1931 The effect of night on milk production. J. Dairy Res., vol. 3, p. 52.

———— 1932 The effect of 9, 12, and 15 hour intervals during the day and night on milk production. J. Dairy Res., vol. 4, p. 28.

CLOTHIER, R. W. 1922 Seasonable variation in the butter-fat content of milk in southern Arizona. Exp. Sta. Rec., vol. 46, p. 679.

COCKEFAIR, E. A. 1928 The activity of the mammary gland, as determined by analysis of mammary blood before and after milking. J. Dairy Sci., vol. 11, p. 230.

CORNER, G. W. 1930 The hormonal control of lactation. I. Non-effect of the corpus luteum. II. Positive action of extracts of the hypophysis. Am. J. Physiol., vol. 95, p. 43.

FELLNER, O. O. 1931 Zur Theorie der Milchsekretion. Klin. Wochenschr., Bd. 10, S. 1169.

GOWEN, J. W. 1924 Milk secretion. Williams & Wilkins, Baltimore, Md.

GRÜTER, F., AND P. STRICKER 1929 Über die Wirkung eines Hypophysenvorderlappenhormones auf die Auslösung der Milchsekretion. Klin. Wochenschr., Bd. 1, S. 2322.

HAYS, W. P. 1926 The effect of environmental temperature on the percentage of fat in cow's milk. J. Dairy Sci., vol. 9, p. 219.

MCEWEN, J. W. G, AND V. E. GRAHAM 1933 A study of butter-fat variations in cow's milk. Sci. Agric., vol. 13, p. 324.

MAGEE, H. E. 1924 Studies in the metabolism of the ruminant by indirect colorimetry. II. The influence of pregnancy on the energy exchange of the goat. J. Agric. Sci., vol. 14, p. 516.

MEIGS, E. B., N. R. BLATHERWICK AND C. A. CARY 1919 Contributions to the physiology of phosphorus and calcium metabolism as related to milk secretion. J. Biol. Chem., vol. 37, p. 1.

NELSON, W. O., AND J. J. PFIFFNER 1931 Studies on the physiology of lactation. I. The relation of lactation to the ovarian and hypophyseal hormones. Anat. Rec., vol. 51, p. 51.

PETERSEN, W. E., L. S. PALMER AND C. H. ECKLES 1929 The synthesis and secretion of milk fat. I. The time of milk and fat secretion. II. An analytical study of the fat of the bovine mammary gland. III. A study of the activity of the perfused surviving gland. With special reference to the fat. Am. J. Physiol., vol. 90, p. 573.

RAGSDALE, A. C., AND C. W. TURNER 1922 The seasonal variations of the per cent of fat in cow's milk. J. Dairy Sci., vol. 5, p. 544.

TURNER, C. W. 1931 The development of the mammary gland as indicated by the inanition and increase in the yield of secretion. Mo. Agric. Exp. Sta. Res. Bull. 156.

TURNER, C. W., AND A. H. FRANK 1930 The effect of the estrus producing hormone on the growth of the mammary gland. Mo. Agric. Exp. Sta. Res. Bull. 145.

———— 1931 The relation between the effect of the estrus producing hormone and a corpus luteum extract on the growth of the mammary gland. Science, vol. 73, p. 295.

TURNER, C. W., A. H. FRANK, C. H. LOMAS AND C. W. NIBLER 1930 A study of the estrus producing hormone in the urine of cattle during pregnancy. Mo. Agric. Exp. Sta. Res. Bull. 150.

TURNER, C. W., AND W. U. GARDNER 1931 The relation of the anterior pituitary hormones to the development and secretion of the mammary gland. Mo. Agric. Exp. Sta. Res. Bull. 158.

TURNER, C. W., AND I. S. SLAUGHTER 1930 The physiological effect of pituitary extract (posterior lobe) on the lactating mammary gland. J. Dairy Sci., vol. 13, p. 8.

WEAVER, E., AND C. A. MATTHEWS 1928 The influence of temperatures and certain other factors upon the percentage of fat in milk. Iowa Agric. Exp. Sta. Res. Bull. 107.

A COMPARATIVE STUDY OF THE SPECIFIC DYNAMIC ACTION OF THE AMINO-ACIDS ALANINE AND GLYCINE[1]

CHARLES M. WILHELMJ

Department of Physiology, Creighton University School of Medicine, Omaha, Nebraska

ONE FIGURE

(Received for publication June 15, 1933)

The work of Lusk and his associates was preeminent in demonstrating the remarkable constancy of the specific dynamic action of certain amino-acids under carefully controlled, standard conditions. Lusk suggested that the constancy of the response entitled it to be classified with certain other physiologic constants, such as pulse rate, body temperature, blood pressure and so forth. Many careful workers in the field of nutrition agree with Lusk regarding the constancy of the response, and in view of these facts it seemed desirable to analyze the specific dynamic actions of the amino-acids, alanine and glycine, as obtained by several independent workers and to establish, if possible, a satisfactory method for expressing the specific dynamic action which would also permit comparison of experiments performed in different laboratories.

In attempting to compare the results obtained in different laboratories, several factors must be taken into consideration. Granting that the accuracy of the respective methods employed, and the care taken in training the animals are practically identical, the single most important variable factor is the

[1] An analysis of work done in the Section of Clinical Metabolism and the Division of Experimental Medicine, The Mayo Clinic, Rochester, Minnesota.

dietary regimen and the nutritional condition of the animals employed. Wilhelmj and Mann ('30) demonstrated that the specific dynamic action of the same amino-acid when administered to the same dog can be markedly altered by a period of total fasting or by total fasting followed by the administration of foods high in carbohydrate. Their experiments represent extreme conditions, but we have some evidence which appears to indicate that changes in the nutritional level at which the animals are in equilibrium can also bring about less pronounced but rather definite changes in the specific dynamic action of alanine and glycine. Because of these facts it appears necessary to limit the comparison to laboratories in which the dietary regimen and hence the nutritional conditions of the animals are approximately the same. The diet used in Rapport's laboratory and in mine was practically the same and was patterned after that described and used by Lusk. It thus appeared that the results from these three laboratories could be used in this comparative study.

The next point to be considered is the manner in which the specific dynamic actions of the same or different amino-acids can be expressed and compared. If the dogs employed are of approximately the same size and therefore have a basal heat production of approximately the same value, and if in addition, approximately the same quantity of amino-acid has been given, it is possible to compare the extra heat production during a given period after administration of the amino-acid with the basal heat production during the same period, and to express the specific dynamic action in percentage of the basal heat production. If, however, one attempts to compare the specific dynamic action in a 7-kilo dog receiving 3.7 gm. of glycine and in an 18-kilo dog receiving 9.5 gm. glycine and a 12-kilo dog receiving 30 gm. alanine and if, in addition, the duration of the respective experiments is 4 hours, 4 hours and 7 hours, the numerous variable factors become confusing and this method of comparison becomes difficult, if not impossible, to apply. The same percentage elevation may also represent a totally different actual value for the specific dynamic

action when the basal values are widely different, and since it is the actual value of the increase, in calories, that is important, this method of expression may fail to give essential and valuable information. Wilhelmj and Bollman ('28) pointed out these and other difficulties in expressing and comparing the specific dynamic actions of amino-acids, and presented evidence which appeared to indicate that the difficulties could be largely overcome by expressing the specific dynamic action as calories of 'extra' heat produced for each mol, or preferably, for each millimol, of amino-acid deaminized. Employing this method of expression I have taken a series of experiments from papers by Lusk, Rapport and Beard, Wilhelmj and Bollman, and Wilhelmj and Mann and selected experiments from the paper of Wilhelmj, Bollman and Mann, besides a series of previously unpublished experiments and have compared them on this basis.

These experiments are shown in table 1 in which fifty-one experiments are tabulated. In the seventh column the results have been expressed as calories of specific dynamic action for each millimol of alanine or glycine, deaminized, and it is apparent that the first six experiments taken from the paper of Wilhelmj and Bollman and of Rapport and Beard, although agreeing fairly well, average almost twice as high as the average values in the remaining forty-two experiments. Experiment 41 dog 3, from Lusk; experiment 11 dog 1, and experment 3 dog 3, of previously unpublished experiments, are in poor agreement with the remaining experiments in their respective groups. These discrepancies will be referred to later; the three experiments have neither been included in the average figures for the respective groups in which they occur, nor in the total average figure.

From the results of unpublished experiments planned to investigate the higher values obtained in the experiments of Wilhelmj and Bollman and Rapport and Beard, it may be stated tentatively and with due caution that it appears that this difference is not merely chance but is of true metabolic significance, and is possibly the result of a difference in the

TABLE 1

Analysis of fifty-one experiments

INVESTIGATOR	AMINO-ACID ADMINISTERED	GRAM	NITROGEN, GM., FROM AMINO-ACID DEAMINIZED	AMINO-ACID DEAMINIZED, PER CENT	AMINO-ACID DEAMINIZED, MILLIMOL	TOTAL SPECIFIC DYNAMIC ACTION, CALORIES	SPECIFIC DYNAMIC ACTION PER MILLIMOL AMINO-ACID DEAMINIZED	LENGTH RESPIRATORY EXPERIMENT, HOURS	DOG WEIGHT, KG.	BASAL HEAT PRODUCTION, CALORIES PER HOUR	REMARKS	DOG
Wilhelmj and Bollman ('28)	Glycine intravenously	5.56	0.437[1]	42	31	15.2	0.49	4.33	10.4	21.8	Exp. 2A	
	Glycine intravenously	6.20	0.348[1]	30	25	10.4	0.41	3.95	11.4	18.0	Exp. 5	
	Alanine intravenously	10.43	0.607[1]	37	43	13.5	0.31	4.18	16.4	23.7	Exp. 6	
	Alanine intravenously	10.24	0.564[1]	35	40	15.3	0.39	4.00	16.1	24.8	Exp. 1A	
					Average of all = 0.40							
					Average glycine = 0.45							
					Average alanine = 0.35							
Rapport and Beard ('27)	Glycine orally	10.0	0.462[2]	25	33	11.2	0.34	3.00	6.5	12.4	Exp. 65	
	Glycine orally	15.0	0.652[2]	23	46	17.3	0.38	4.00	6.5	12.4	Exp. 66	
					Average = 0.36							
Wilhelmj and Mann ('30)	Glycine intravenously	5.56	0.614[1]	59	44	7.9	0.18	4.02	10.3	13.4	Exp. 2	1
	Glycine intravenously	5.56	0.478[1]	46	34	6.3	0.19	4.12	10.4	13.6	Exp. 3	1
	Glycine intravenously	5.08	0.483[1]	47	32	8.5	0.27	4.00	9.5	11.6	Exp. 4	1
	Glycine intravenously	5.08	0.684[1]	72	49	10.7	0.22	4.05	9.4	12.0	Exp. 5	1
	Glycine intravenously	3.69	0.490[1]	71	35	7.8	0.22	4.02	6.9	11.7	Exp. 1	3
	Glycine intravenously	3.69	0.547[1]	79	38	7.4	0.19	4.02	7.0	12.1	Exp. 2	3
	Alanine intravenously	8.54	0.279[1]	21	20	5.0	0.25	4.00	13.4	17.6	Exp. 1	2
	Alanine intravenously	8.54	0.463[1]	35	33	5.5	0.17	4.03	13.4	16.7	Exp. 2	2
	Alanine intravenously	8.54	0.521[1]	39	37	6.0	0.16	4.08	13.5	17.6	Exp. 3	2
	Alanine intravenously	8.54	0.623[1]	46	44	7.1	0.16	4.05	13.6	17.8	Exp. 4	2
Wilhelmj, Bollman and Mann ('31)	Glycine intravenously	8.18	0.840[1]	55	60	16.0	0.27	4.13	15.7	19.1		1
	Glycine orally	8.18	1.040[1]	68	74	16.2	0.22	3.95	15.9	20.0		1
	Glycine intravenously	5.83	0.484[1]	44	35	8.8	0.25	4.03	10.9	15.1		3
	Glycine intravenously	5.83	0.662[1]	61	47	10.8	0.23	4.02	10.9	15.5		3
	Glycine orally	10.00	1.030[1]	55	73	16.3	0.22	4.03	11.2	16.8		3
					Average of all = 0.21							
					Average glycine = 0.22							
					Average alanine = 0.19							
Lusk ('12)	Glycine orally	25.0	1.98[1]	43	144	31.3	0.22	8.00	9.6	16.2	41[2] Exp. 48 41[2]	
	Glycine orally	25.0	2.12[1]	45	150	28.5	0.19	8.00	9.6	16.2	Exp. 52	
Lusk ('15)	Glycine orally	20.0	2.38[3]	64	171	34.0	0.20	6.00	12.6	16.7	Exp. 56	3
	Glycine orally	20.0	2.53[3]	68	181	33.5	0.19	6.00	12.7	16.7	Exp. 57	3
	Glycine orally	10.0	1.29[3]	69	92	17.5	0.19	6.00	12.8	16.7	Exp. 59	3
	Glycine orally	10.0	1.25[3]	67	89	15.9	0.18	6.00	12.9	16.7	Exp. 61	3
Lusk ('12)	Alanine orally	20.0	0.801[1]	26	58	12.6	0.22	4.00	9.7	16.2	Exp. 43	3
Lusk ('15)	Alanine orally	20.0	1.08[3]	34	76	24.0	0.32[4]	5.00	12.4	16.7	Exp. 41	3
	Alanine orally	20.0	1.39[3]	44	99	17.2	0.17	6.00	12.4	16.7	Exp. 43	3
	Alanine orally	30.0	2.07[3]	44	148	29.3	0.20	6.00	12.3	16.7	Exp. 44	3
					Average of all = 0.20							
					Average glycine = 0.20							
					Average alanine = 0.20							

TABLE 1—*Continued*

INVESTIGATOR	AMINO-ACID ADMINISTERED	GRAM	NITROGEN, GM., FROM AMINO-ACID DEAMINIZED	AMINO-ACID DEAMINIZED, PER CENT	AMINO-ACID DEAMINIZED, MILLIMOL	TOTAL SPECIFIC DYNAMIC ACTION, CALORIES	SPECIFIC DYNAMIC ACTION PER MILLIMOL AMINO-ACID DEAMINIZED	LENGTH RESPIRATORY EXPERIMENT, HOURS	DOG WEIGHT, KG.	BASAL HEAT PRODUCTION, CALORIES PER HOUR	REMARKS	DOG
Unpublished	Glycine intravenously	9.47	1.060[1]	60	76	13.8	0.18	4.05	17.7	19.6	Exp. 1	1[5]
	Glycine intravenously	9.47	0.939[1]	53	67	13.4	0.20	4.05	17.7	20.1	Exp. 2	
	Glycine intravenously	9.47	0.989[1]	56	71	15.2	0.21	4.05	17.9	20.3	Exp. 3	
	Glycine intravenously	9.47	0.953[1]	54	68	14.2	0.21	4.07	17.7	19.8	Exp. 4	
	Glycine intravenously	9.47	1.110[1]	63	79	13.9	0.18	4.03	17.7	20.3	Exp. 5	
	Glycine intravenously	9.47	1.120[1]	63	80	12.9	0.16	4.05	17.8	20.7	Exp. 6	
	Glycine intravenously	9.47	0.722[1]	41	51	11.4	0.22	4.05	18.0	20.4	Exp. 7	
	Glycine intravenously	9.47	0.774[1]	44	55	11.2	0.20	4.05	17.9	21.0	Exp. 8	
	Glycine intravenously	9.47	0.819[1]	46	58	9.7	0.17	4.05	18.0	20.8	Exp. 9	
	Glycine intravenously	9.47	0.872[1]	49	62	10.0	0.16	4.03	18.2	21.6	Exp. 10	
	Glycine intravenously	9.47	0.667[1]	38	48	14.9	0.31[4]	4.08	17.8	19.6	Exp. 11	
Unpublished	Glycine intravenously	5.51	0.577[1]	56	41	8.9	0.22	4.05	10.0	14.7	Exp. 1	3[5]
	Glycine intravenously	5.51	0.830[1]	81	59	13.3	0.23	4.05	10.0	14.4	Exp. 2	
	Glycine intravenously	5.51	0.837[1]	81	60	8.2	0.14[4]	4.05	10.2	15.1	Exp. 3	
	Glycine intravenously	5.51	0.843[1]	82	60	10.6	0.18	4.05	9.9	14.7	Exp. 4	
	Glycine intravenously	5.51	0.735[1]	71	52	9.7	0.19	4.03	10.1	14.8	Exp. 5	
	Glycine intravenously	5.51	0.567[1]	55	40	9.1	0.23	4.05	10.2	15.6	Exp. 6	
	Glycine intravenously	5.51	0.838[1]	81	60	10.2	0.17	4.05	10.2	14.3	Exp. 7	
	Glycine intravenously	5.51	0.683[1]	66	49	7.9	0.16	4.05	10.3	14.8	Exp. 8	
	Glycine intravenously	5.51	0.783[1]	76	56	9.0	0.16	4.03	10.1	14.8	Exp. 9	

Average = 0.19

Average of forty-two experiments = 0.20

Average of thirty-five experiments with glycine = 0.20

Average of seven experiments with alanine = 0.19

[1] Calculated from the actual increase in urea plus ammonia nitrogen over the basal value.

[2] Calculated from the increase in total nitrogen above the total nitrogen of the basal period. The figure thus obtained therefore includes the amount of amino-acid excreted unchanged, but since this is usually small in amount the error would not be significant.

[3] In comparing the increase in heat production hour by hour with the rate of deaminization of the amino-acids, Lusk used percentage values obtained by Csonka based on the excretion of extra glucose in phlorhizinized dogs, and assumed that the hourly rate of deaminization in these experiments would be approximately the same. However, he also gives the actual values for extra urea plus ammonia nitrogen obtained from these dogs when the same quantity of amino-acid was administered on days when the animals were not in the calorimeter. The latter, actual values, rather than assumed values have been used although there was surprisingly good agreement between the results obtained with either set of figures.

[4] Not included in averages of respective groups or in final averages.

[5] Experiments 44 and 48 as well as 41 and 52 were performed separately, but each pair can be considered as representing one experiment, since 44 and 41 cover the first 4 hours after administering 25 gm. of glycine and 48 and 52 cover the second 4-hour period, after giving the same quantity of glycine to the same dog. Each pair has been calculated as one experiment since there seems to have been in both instances a lag in elimination of extra urea during the first 4-hour period after administering glycine, and a corresponding increase during the second 4-hour period. If the experiments are calculated separately, the value of the specific dynamic action per millimol of glycine deaminized, as calculated from the elimination of extra urea, is 0.41 and 0.49 calorie for experiments 41 and 44 respectively, and 0.10 and 0.11 calorie for experiments 52 and 48, respectively.

level or plane of nutrition of the animals used. The animals used by Wilhelmj and Bollman were on a different level of nutrition from that of the remaining animals, and shortly after the level of nutrition was changed, lower figures were obtained. For the present these six experiments will be omitted from consideration in the text, and the analyses will be confined to the remaining forty-two experiments.

There was a rather remarkable constancy of the results in these forty-two experiments when expressed as calories of specific dynamic action for each millimol of amino-acid deaminized. The average value in the fifteen experiments from the papers of Wilhelmj and Mann, and Wilhelmj, Bollman and Mann is 0.21 calorie per millimol; in the nine experiments of Lusk the average is 0.20 calorie per millimol, and in the eighteen previously unpublished experiments the average was 0.19 calorie per millimol. The average value for all forty-two experiments was 0.20 calorie per millimol. The separate averages for alanine and glycine in these groups were also almost identical, and showed clearly that when expressed in this manner there was no essential difference between the specific dynamic action of these two amino-acids. A more detailed analysis of the experiments makes this constancy appear significant, in view of the following variations: 1) the weights of the dogs employed varied from 6.9 to 18.2 kilo with basal levels of heat production between 11.7 and 21.6 calories per hour; 2) the duration of the experiments varied from 3.95 hours to 8 hours, and 3) the amounts of alanine or glycine administered varied from 3.69 to 30 gm., and were given intravenously in thirty-one experiments and orally in eleven experiments. Concerning these particular factors it would be rather difficult to arrange for a much greater degree of dissimilarity.

When the relationships between the quantities of amino-acids administered, the total calories of specific dynamic action and the specific dynamic action in calories per millimol of amino-acid deaminized were analyzed, the following relations were found: 1) the maximal and minimal quantities of

amino-acid administered varied in a ratio of 1:8.1 (3.69 to 30 gm.); 2) the maximal and minimal values for the total specific dynamic actions varied in a ratio of 1:6.8 (5 to 34 calories), the low specific dynamic action of 5 calories being obtained with 8.54 gm. of alanine given intravenously and the high value of 34 calories with 20 gm. of glycine given orally, the specific dynamic actions per millimol of amino-acid deaminized in these two experiments were 0.25 and 0.20 calorie respectively (a ratio of 1:1.3), and 3) the maximal and minimal values for the specific dynamic action per millimol of amino-acid deaminized varied in a ratio of 1:1.7 (0.16 to 0.27 calorie). From this brief analysis it is obvious that widely different values for the total calories of specific dynamic action resulting from the administration of markedly different quantities of amino-acid, gave reasonably constant values when expressed as calories of specific dynamic action for each millimol of amino-acid deaminized.

The total specific dynamic action of alanine and glycine in the forty-two experiments described bears a direct linear relationship to the amount of amino-acid deaminized (fig. 1). In these forty-two experiments the quantities of amino-acid deaminized varied from 20 to 184 m.Mol. The experiments were divided into groups, each group increasing by 15 m.Mol., and the average figure in each group for the millimols of amino-acid deaminized was plotted against the corresponding average value for the total specific dynamic action. The line was constructed by multiplying the average figure for millimols of amino-acid deaminized in each group by 0.20 calorie, which is the average value of the specific dynamic action per millimol of amino-acid deaminized in the forty-two experiments. It is seen that the average values for the eight groups of experiments followed this line very closely and justified the conclusion that, within the limits of experimental error, the specific dynamic action of alanine and glycine was a linear function of the quantity of amino-acid deaminized, and that for these two amino-acids the specific dynamic action per millimol of amino-acid deaminized was approximately 0.20 calorie.

Comment

Lusk ('15) showed that the specific dynamic action of glycine is, in general, proportional to the amount administered, and further that the hours of greatest increase in heat production after giving glycine are coincident with the hours of the greatest metabolism of glycine. Chambers and Lusk

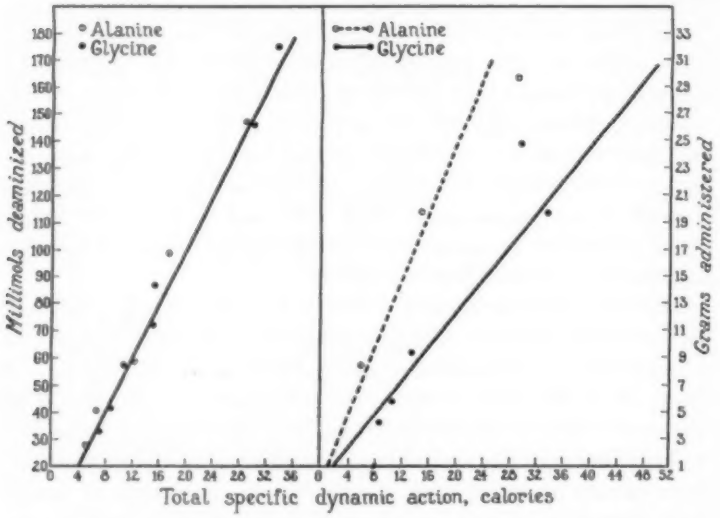


Fig. 1 Chart showing the linear relationship of total specific dynamic action to the millimols of amino acid deaminized. The second figure shows the same line calculated for alanine and glycine on the basis of the number of grams administered.

('30) showed that with glycine the total specific dynamic action is independent of the size of the dog since the extra calories resulting from the ingestion of 10 gm. of glycine were the same in two dogs weighing 6.6 and 12.7 kilo. The data reported in this paper are in general a confirmation of these findings of Lusk, and they, in turn, appear to lend strength to the statement that the specific dynamic action of alinine and glycine is proportional to and can be expressed and com-

pared as extra calories of heat production per millimol deaminized. The proportionality of the total specific dynamic action to the total amount of amino-acid administered appears to depend on the quantity of the amount administered, which was deaminized; under ordinary conditions, of course, the amount deaminized increases as larger quantities are administered, but the proportionality appears to be closer when the specific dynamic action is compared with the amount deaminized rather than with the total amount given (table 1).

From the analysis recorded in this paper it appears that the specific dynamic action of alanine and glycine is practically identical when expressed as calories of extra heat produced per millimol of amino-acid deaminized. On the basis of many careful experiments Lusk, and Chambers and Lusk stated that the specific dynamic action of glycine is practically twice that of alanine, that is, for glycine approximately 100 per cent of the physiologically available calories appeared as extra heat, constituting the specific dynamic action, whereas for alanine approximately 50 per cent of the physiologically available calories appeared as extra heat of the specific dynamic action. Although these statements appear to be contradictory, a more detailed analysis shows that they are in reality the same. Lusk calculated that the physiologically available calories per gram of glycine, when combusted in the body are 2.099, and per gram of alanine 3.549 calories. Taking the molecular weight of glycine as 75 and of alanine as 89 it is found that the physiologically available calories per millimol of glycine are 0.16 and for alanine 0.32, therefore, if as Lusk found, 100 per cent of the physiologically available calories in glycine and 50 per cent in alanine appear as specific dynamic action, then when expressed as calories per millimol of amino-acid the values for alanine and glycine will be the same and will amount to 0.16 calorie. This value is very close to the average value of 0.20 calorie which was found for the forty-two experiments analyzed in table 1.

When the specific dynamic action of alanine and glycine is expressed as calories per millimol of amino-acid deaminized,

certain of the difficulties inherent in the other methods of expression appear to be eliminated and it is possible to compare the results obtained in different laboratories employing different methods, different sized dogs, and widely different quantities of amino-acids, provided certain factors, especially the nutritional conditions of the animals employed in the different laboratories, are maintained on a reasonably comparable basis. It is important to emphasize that in order to obtain such constancy of results it is essential that an equilibrium should exist between the amount of urea produced by deaminization of the amino-acid and the amount of extra urea eliminated in the urine; any factor, for instance, which produced diuresis not dependent on and proportional to that accompanying the elimination of the urea formed by deaminization of the amino-acid, may by washing out urea not concerned in this equilibrium, prevent the true values from being obtained. On the other hand, any factor which reduces the ability of the kidneys to eliminate urea or increases the avidity of the tissues for holding urea, will produce errors in the opposite direction. It is possible that Lusk's experiment 41 and experiments 11 and 3 in the series of those previously unpublished, which are in rather poor agreement with the remaining experiments in their respective groups, were complicated by some such disturbance in this equilibrium. Although the values obtained in the remaining forty-two experiments are reasonably constant, it is likely that still better agreement will be obtained when other secondary factors are known and can be controlled.

There are at present no adequate explanations or hypotheses which will account for the identical value of the specific dynamic action of alanine and glycine when expressed as calories per millimol deaminized, or the constancy of the value under almost identical conditions of nutrition. At the Thirteenth International Physiological Congress, Lusk presented important data which were published by Chambers and Lusk ('30). These data were based on calculations by Adams ('26). Adams calculated that the laws of thermo-

dynamics necessitate the expenditure of certain quantities of energy in order to transform alanine and glycine into glucose and urea according to the equations of Lusk, whereas the same type of calculation when applied to glutamic acid, which was shown by Lusk ('12) and by Chambers and Lusk ('30) to exert no specific dynamic action, showed that the change to glucose and urea could be accomplished without the expenditure of energy. Adams' figures showed that to change 1 gm. of glycine to glucose and urea requires the expenditure of 0.871 calorie, whereas for alanine the corresponding value is 0.337 calorie. When these values are expressed as the energy necessary to bring about the reaction for 1 m.Mol. of amino-acid, the energy amounts to 0.067 calorie for glycine and 0.031 calorie for alanine; comparing these figures with the values for the specific dynamic actions per millimol of amino-acid deaminized in the animal body, it is seen that the actual specific dynamic action of glycine is approximately three times and of alanine approximately six times the minimal energy necessary to convert these two amino-acids to glucose and urea. Concerning these values Chambers and Lusk stated:

> These are minimal and, just as external muscular work may require a three-fold energy production for its accomplishment, so this intermediary chemical work may be associated with the production of free heat. The figures given by Adams indicate a minimal energy quantum of 10 per cent of that physiologically available in 1 gm. of alanine, in order to drive it to glucose and urea, whereas the specific dynamic action of alanine is 50 per cent of the available calories in the material. In the case of glycine the minimal quantum of energy necessary to drive it to glucose and urea is 41 per cent of the available calories in the material whereas the specific dynamic action of glycine as measured by the liberation of extra heat in metabolism is 100 per cent . . . . This work of Adams explains in part, if not wholly, the specific dynamic effect of protein in the simplest way.

It is essential to emphasize that in expressing the specific dynamic action of alanine and glycine as calories per millimol of amino-acid deaminized, I do not wish to imply that the

specific dynamic action must necessarily be due to the process of deaminization per se. The studies of Lusk and his associates, which were confirmed by Aub, Everett and Fine ('27), have shown clearly that glutamic and aspartic acids may be deaminized without appreciably elevating heat production. On the other hand, the work of Wilhelmj, Bollman and Mann demonstrated that in totally dehepatized dogs, in which intravenously administered amino-acids are not deaminized, there is no specific dynamic action from alanine or glycine. These observations, in conjunction with the fact that there is a greater constancy in the specific dynamic action when it is correlated on the basis of millimols of amino-acid deaminized than on the basis of grams administered, suggests that the major part of the reactions concerned in its production occurs, under the condition of these experiments, after deaminization has taken place. In the totally fasting animal, on the other hand, there is the possibility that a certain proportion of the extra heat may arise without previous deaminization of the amino-acid, and be the result of anabolic processes by which the amino-acids are built into more complex nitrogen-containing compounds. The interesting experiments of Kiech and Luck ('31) appear to give direct evidence of such a process. Undoubtedly the intermediate steps of the metabolism of amino-acids or other substances will vary, depending on whether the animal is overnourished or undernourished, and in consequence not only is it possible for the magnitude of the specific dynamic action to vary under different nutritional conditions, but different reactions will necessarily be concerned in its production.

SUMMARY

A series of fifty-one experiments performed in three independent laboratories have been reviewed. Forty-two of these experiments appear to be comparable and have been subjected to a detailed analysis. It has been found that when the specific dynamic actions of alanine and glycine are expressed as calories per millimol of amino-acid deaminized,

the values are practically identical if animals are on approximately the same nutritional level. The average value for the specific dynamic action per millimol of amino-acid deaminized in these forty-one experiments was 0.20 calorie. The average of eight experiments in which alanine was employed gave a value of 0.19 calorie per millimol deaminized, while thirty-four experiments with glycine gave an average value of 0.20 calorie. This close agreement of the values when expressed as calories of specific dynamic action per millimol of amino-acid deaminized was obtained with dogs ranging in weight from 6.9 to 18.2 kilos and when amounts of amino-acid ranging between 3.69 gm. of glycine and 30 gm. of alanine, which gave total specific dynamic action of from 5 to 34 calories. The amino-acids were given orally in eleven experiments and intravenously in thirty-one experiments.

This analysis shows clearly that comparable results can be obtained in different laboratories employing different methods, different sized dogs and widely different quantities of amino-acids, provided that the dietary and environmental conditions of the animals are approximately the same.

LITERATURE CITED

ADAMS, E. T. 1926 Specific dynamic action from the standpoint of the second and third laws of thermodynamics. J. Biol. Chem., vol. 67, p. 21.

AUB, J. C., M. R. EVERETT AND J. FINE 1927 The intravenous administration of amino acids to decerebrate and urethanized cats. Am. J. Phys., vol. 79, p. 559.

BOLLMAN, J. L., F. C. MANN AND T. B. MAGATH 1926 Studies on the physiology of the liver. XV. The effect of total removal of the liver on deamination. Am. J. Phys., vol. 78, p. 258.

CHAMBERS, W. H., AND G. LUSK 1930 Animal calorimetry. XXXIX. Specific dynamic action in the normal and phlorhizinized dog. J. Biol. Chem., vol. 85, p. 611.

KIECH, V. C., AND J. M. LUCK 1931 Amino acid metabolism. I. The relative rates of amino acid disappearance and urea formation. J. Biol. Chem., vol. 94, p. 433.

LUSK, G. 1912 Animal calorimetry. V. The influence of the ingestion of amino acids upon metabolism. J. Biol. Chem., vol. 13, p. 155.

———— 1915 Animal calorimetry. XI. An investigation into the causes of the specific dynamic action of the foodstuffs. J. Biol. Chem., vol. 20, p. 555.

RAPPORT, D., AND H. H. BEARD 1927 The effect of protein split-products upon metabolism. I. The fraction extracted by and precipitated in butyl alcohol. J. Biol. Chem., vol. 73, p. 285.

WILHELMJ, C. M., AND J. L. BOLLMAN 1928 Specific dynamic action and nitrogen elimination following intravenous administration of various amino acids. J. Biol. Chem., vol. 77, p. 127.

WILHELMJ, C. M., J. L. BOLLMAN AND F. C. MANN 1928 Studies on the physiology of the liver. XVII. The effect of removal of the liver on the specific dynamic action of amino acids administered intravenously. Am. J. Phys., vol. 87, p. 497.

———— 1931 A study of certain factors concerned in the specific dynamic action of amino acids administered intravenously and a comparison with oral administration. Am. J. Phys., vol. 98, p. 1.

WILHELMJ, C. M., AND F. C. MANN 1930 The influence of nutrition on the response to certain amino acids. I. The effect of fasting. Am. J. Phys., vol. 93, p. 69.

———— 1930 The influence of nutrition on the response to certain amino acids. II. The effect of fasting followed by diets high in carbohydrates. Am. J. Phys., vol. 93, p. 258.

———— 1931 A study of certain factors concerned in the specific dynamic action of amino acids administered intravenously and a comparison with oral administration. Am. J. Phys., vol. 98, p. 1.

NEUROLOGICAL DISTURBANCES IN RATS REARED ON DIETS DEFICIENT IN VITAMIN A[1]

S. B. D. ABERLE

Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven

ONE PLATE (EIGHT FIGURES)

(Received for publication July 5, 1933)

In the course of experiments designed to study the reproductive organs in rats fed diets low in vitamin A, it was found that certain of the animals developed a marked weakness of the extremities. The weakness was manifested only in those animals which had been on the diet long enough to bring the weight increment to zero, or to develop xerophthalmia. Certain questions arose as to the relation of this condition to other symptoms of avitaminosis of A: 1) what was the incidence of the paralysis; 2) what was its temporal relation to other symptoms such as continual cornified vaginal cells, xerophthalmia and loss of weight; 3) what effect loss of weight alone has upon the nervous system; 4) what relation the age of the animal and the amount of stored vitamin have to the onset of the paralysis; 5) what neurological lesions were present; and 6) whether vitamin A was effective in curing this condition.

EXPERIMENTAL METHODS

The experimental rats were obtained from a stock in which the care and feeding had been carefully standardized. Breeding females were mated at about 100 days of age. All litters were reduced to six on the day of parturition. During preg-

[1] This investigation was partially subsidized by a grant from the National Research Council Committee for Research in Problems of Sex.

nancy and lactation the mothers of all the rats in group I and some of the rats in group III were fed 100 per cent calf-meal,[2] which is low in vitamin A. The other breeding females were fed table scraps, which included carrots, greens, cereals and milk, but no additional A was given.

Five groups of rats comprising forty-three animals were used. Each experimental rat was isolated in a wire-mesh cage. A wire base allowed feces to drop through. Partitions were kept between cages to prevent the spread of infection. The water bottles and tubes were washed and sterilized twice a week, and cages were sterilized weekly. Rubber gloves were worn and dipped in lysol after each animal had been handled. The animals were weighed twice a week. Fresh food was kept constantly in the cage.

It was possible to prolong the lives of rats with severe avitaminosis by keeping the room warm. The temperature was kept between 80° and 84°F. When the animals were about to die, they were sacrificed in order to preserve the tissues while fresh and each was autopsied. The gross pathological lesions were recorded, and the tissues prepared for microscopic study. The brachial plexuses, sciatic and vagus nerves were dissected out and fixed in Müller's fluid and in 10 per cent formalin. The spinal cords and brain were fixed in 95 per cent alcohol. Dr. Harry M. Zimmerman ('33) studied these materials; the result of his observations which have been published need be only briefly summarized here. His diagnosis is based on sections stained with the original Nissl method, Spielmeyer's stain, Scharlach R and osmic acid.

[2] Calf-meal is a commercial mixture containing approximately the following ingredients in 100 parts; oil meal 15, malted barley 10, whole wheat 22, oat flour 15, dried skim milk 15, yellow corn meal 20, ground limestone 1, steamed bonemeal 1, salt 1.

Diet. The composition of the vitamin A deficient diet was:

Ingredient	*Per cent*
Casein [3]	15–18
Cornstarch	56–63
Hydrogenated vegetable oil [4]	18–22
Osborne-Mendel salt mixture IV	4

Because rats ate more when the food was varied the proportions of the diet were changed weekly or biweekly. This was done in the following manner; casein, cornstarch and hydrogenated vegetable oil were changed from 15, 63, 18 per cent to 18, 56, 22 per cent, respectively.

Adequate amounts of vitamins B, D and E were supplied by yeast,[5] irradiation,[6] and hydrogenated vegetable oil. Vitamin C has not been found necessary in the normal metabolism of the rat (Parsons, '20). The tests of the yeast showed that $\frac{1}{10}$ gm. daily was sufficient to produce a gain in weight in rats deprived of vitamin B. The same batch of yeast was used throughout the experiment. Four to eight-tenths of a gram was fed daily to these experimental animals in separate containers. In addition, tiki-tiki[7] and yeast concentrate were fed to some of the rats. A biological test of ergosterol showed that 0.0009 mg. fed over a period of 10 days gives a 2-plus line test. One-thousandth to two-thousandth gram irradiated ergosterol was given daily, if no irradiated yeast was included. With the irradiated yeast it was sometimes omitted. To irradiate the yeast it was spread in thin layers, exposed to the rays of a mercury-vapor lamp at a distance of 15 inches for 15 minutes, then mixed, re-spread, and irradiated for another 15 minutes (Hess, '27).

Evans and Burr ('27) were able to obtain successful reproduction in rats on an E-free diet, by giving 22 per cent of hydrogenated vegetable oil[8] in the diet or by feeding 500 mg. of the same oil during the gestation period.

[3] Eimer and Amend technical casein.

[4] Crisco.

[5] M. H. Givens, of the Northwestern Yeast Company, supplied tested yeast.

[6] Irradiated ergosterol was supplied by the Fleischmann laboratories.

[7] The author is indebted to Dr. George R. Cowgill for supplying tested tiki-tiki and yeast concentrate.

[8] Crisco.

Frequent observations were made in order to record the first appearance of vitamin A deficiency. Vaginal lavage was taken on each rat four to six times a week. The appearance of continued cornified cells in the vagina was found to be the first indication of vitamin A deficiency (Aberle, '33). The rats were watched with similar frequency for the appearance of xerophthalmia or loss of weight. The diagnosis of xerophthalmia was based upon the appearance of red granules in or about the eyes. The failure to gain weight was taken when the weight increment was zero. Incontinence of urine was recorded when it was first noted that the hair about the urinary papilla of the rat was wet and stained. After this, a constant dribbling of urine and a characteristic odor were present.

RESULTS

Group I. Rats on diets deficient in vitamin A, from mothers on diet of 100 per cent calf-meal during pregnancy and lactation. The average age at which they were weaned, and the time of the various manifestations of A deficiency are shown in table 1. The first appearance of the paralysis in this group occurred at the average age of 61.1 days. The symptoms of the paralysis were arbitrarily divided into four stages, in order to facilitate record taking. Each phase was defined as having one outstanding characteristic, although there was variation in the duration and severity of the symptoms.

Stage I showed best when the animal was suspended by the nape of the neck (fig. 2). There was a hyper-extension of the hind legs and a marked curvature of the spine. In the normal rat (fig. 1), the legs are usually flexed when they are removed from the floor. If flexion does not occur normally, it can be induced by stimulation to the sole of the foot. In the rats in stage I slight stimulation such as scraping the sole of the foot with the blunt part of forceps was not sufficient to cause the legs to be flexed. The rat shown in figure 3 had stages I and II of the paralysis. During stage I the legs of the experimental rats spread farther apart in walking than those of the normal animal and there was an awkwardness in the movements.

TABLE 1

Age and weight[1] of rats at the appearance of various symptoms of avitaminosis of A

	WEANED AND DIET STARVED	CONTINUAL CORNIFIED SMEAR	XEROPHTHALMIA	WEIGHT-GAIN STOPPED	INCONTINENCE	PARALYSIS STAGE [2]				C.L.O.	DIED	KILLED	PARALYSIS DISAPPEARED
						I	II	III	IV				
Group I. Rats on vitamin A deficient diet from mothers on diet of 100 per cent calf meal during pregnancy and lactation													
Number of rats	12	12	10	10	8	9	5	8	4		6	6	
Mean age (days)	22.0	46.5	58.6	64.1	68.1	61.1	62.6	66.8	81.8		78.3	74.7	
S.D.M.	0.3	2.0	0.8	3.0	3.5	1.8	2.3	4.4	2.1		4.5		
Mean weight (grams)	28.3	75.3	88.4	93.4	86.3	87.7	83.6	84.6	89.8		85.7	86.0	
S.D.M.	1.2	4.6	5.5	4.6	4.8	4.5	5.5	4.6	5.4		5.8		
Group II. Rats on vitamin A deficient diet from mothers on table scrap diet													
Number of rats	15	10	9	7		6	6				3	12	
Mean age (days)	21.6	62.7	105.1	111.0		112.7	121.5				75.3	84.9	
S.D.M.	0.2	2.2	3.5	3.5		2.0	2.5						
Mean weight (grams)	35.4	124.5	147.0	156.4		159.7	153.3				65.6	108.1	
S.D.M.	1.8	8.4	9.9	10.5		11.2	13.1						
Group III. Rats allowed to develop neural symptoms and then given cod liver oil													
Number of rats	6	6	5	5	2	1	5	2	2	6	1	5	3
Mean age (days)	21.8	55.3	63.0	62.8	58.5	56.0	68.4	67.5	71.0	76.8	90.0	97.0	96.7
S.D.M.	0.3	3.3	1.9	2.3			2.1			1.7			
Mean weight (grams)	31.5	91.2	95.8	100.4	77.5	60.0	94.4	81.0	79.0	88.7	60.0	149.2	160.0
S.D.M.	1.3	9.3	11.2	10.5			9.0			7.5			

[1] Weight on nearest day, ± 1 to 4 days.

[2] Stage I. Failure to flex hind legs upon suspension. Stage II. Legs slip in walking. Stage III. Sprawling walk. Stage IV. Dragging hind legs.

The next symptoms, characterizing stage II, were noticed when the animal walked. These symptoms appeared at an average age of 62.6 days in this group. There was incoordination of movements. The hind feet, instead of being placed in one position which was maintained until the foot was lifted to take the next step, slipped at each step (figs. 3, 4). When placed in a position to take a step, however, the foot was approximately parallel to the body.

The outstanding symptom for stage III appeared at an average age of 66.8 days. The feet, instead of being held parallel to the body, were extended at an angle of about 45° in standing and in walking (fig. 5). The helplessness of the hind legs can best be seen in the photograph showing a rat with stage II paralysis trying to climb a small wire fence (fig. 8). The hind legs are so weak that they can scarcely be moved. Some of the animals showed a marked degree of spasticity. The control climbed over the fence easily (fig. 7).

Four animals lived to show stage IV of the paralysis. At this time the legs were dragged along helplessly when the animal moved forward (fig. 6). The fore legs showed involvement in only one animal. In general, clumsiness and incoordination appeared first; then, if the condition progressed further, spasticity appeared. Whether or not the sensory loss occurred in the order of muscle sense, touch and pain, cannot be determined from the observations made.

Of the twelve rats from mothers on a calf-meal diet, two died before and one after xerophthalmia appeared. The remaining nine all developed the paralysis. This remained the same or became progressively worse on the A deficient diet. Paralysis occurred at about the same time as xerophthalmia or loss of weight. The clinical symptoms of the paralysis were confirmed by microscopic sections of the cord and peripheral nerves. The traces of vitamin A present in technical casein probably were responsible for their survival after symptoms of avitaminosis had become marked. Five of these animals were given additional tiki-tiki and yeast concentrate, but the extra vitamin B had no effect on their symptoms.

In each of the nine rats lesions were found in the spinal cord consisting of degeneration of the medullary sheaths of the sensory tracts at the periphery. In four of the animals the posterior columns showed degeneration; and in two, the entering posterior nerve roots showed it. These changes were demonstrated by the Spielmeyer myelin-sheath stain and by the Marchi method. In rat 72, paralyzed for the longest period, fatty change was demonstrated in the white matter of the cord by the Scharlach R stain. In rat 65 there was degeneration of the anterior roots in addition to the posterior. The vagi were degenerated in only four of the animals examined; in one they were not examined. Every one of the animals showed extensive degeneration[9] of the medullary sheaths of the brachial plexuses and sciatic nerves.

Group II. Rats on diet deficient in vitamin A from mothers fed a table scrap diet. Although the breeding females were not given additional vitamin A in the form of cod liver oil, they probably had enough to endow the offspring with a considerable store of the vitamin.

Of the nine rats who lived to develop xerophthalmia, six showed stage I or II, or both, of the paralysis. Although at that time their average age and weight were approximately twice that of those in group I, no further symptoms developed. The paralysis did not appear as early, or with the same intensity as group I, which started without as much stored vitamin A in the tissues. The lesions of the peripheral nerves of two animals which were examined, were similar to those in group I. The cord of one rat was studied and found to show degeneration of the posterior columns and nerve roots, the motor tracts and to a less degree the anterior roots.

Group III. Six rats allowed to develop neural symptoms and then given cod liver oil. Two rats came from mothers which had been on 100 per cent calf-meal during pregnancy and lactation. These rats developed all four stages of the paralysis and incontinence of urine. Tiki-tiki or concentrated yeast had no effect on the symptoms. After the symptoms

[9] These lesions are often designated by the term polyneuritis in the literature.

were well established, cod liver oil was administered daily. In one rat, to which cod liver oil was given on the sixty-fifth day of life, xerophthalmia completely disappeared within 12 days, incontinence of urine in 5 days, and the neural symptoms by 90 days of age, or 25 days after cod liver oil therapy. At this time the rat's weight was almost double that at the time therapy was initiated.

In the second rat after xerophthalmia was marked and all the stages of the paralysis were well developed, cod liver oil was given. Eight days after daily feeding of cod liver oil the eyes had cleared up. Thirteen days later, when the animal was killed, the paralysis had improved from stage IV to a II condition.

Three rats (from mothers on a table scrap diet until the thirteenth day of lactation, then placed on 100 per cent calf-meal) developed only stage II of the paralysis. After they had showed symptoms for 4, 5 and 10 days, they were given cod liver oil. Clinical symptoms of the paralysis disappeared after 20 and 22 days in two animals, and after 14 days in one rat which died. One rat from a mother on a table scrap diet did not develop any symptoms of the paralysis.

In the four animals where clinical recovery occurred, and in which the spinal cords and peripheral nerves were examined microscopically, it was found that they contained extensive lesions, essentially the same as those found in group I. The clinical improvement, therefore, must be due entirely to repair in the tissues not demonstrated by the technique used. This clinical improvement with neural lesions shows the possibility of having no demonstrable clinical symptoms in the pressure of extensive degeneration within the central nervous system.

Group IV. Rats kept on a table scrap diet, then given a limited amount of food. Four rats were kept on a table scrap diet until 50 days of age. They were then given 1 gm. daily of the purified food and vitamins as given to the rats on the A deficient diet, and in addition $\frac{1}{2}$ cc. of cod liver oil. Their loss of weight on this restricted diet paralleled that of the rats on the A deficient diet. They died from 10 to 17 days after

being put on the experimental diet, mean weight 64.5 gm. They showed no symptoms of paralysis. They were weak and exceedingly irritable and had rough coats, but the feet were held in the usual position, both when the rats were extended by the nape of the neck and when they walked. In two animals the nervous tissue was examined. No degeneration was found in the cords. All the peripheral nerves were free of degeneration except for one small zone in the nerve of one animal where, by the use of Marchi preparations, a few black granules were found. In group I the average life of two of the three rats, which showed clinical or microscopic evidence of the paralysis, was 13.5 days after they commenced losing weight. This length of life shows that the loss of weight alone did not account for the paralysis.

Group V. Controls. The control group contained a total of six animals, five from mothers on table scrap diets and one from a mother on 100 per cent calf-meal during pregnancy and lactation. Four were given the purified diet with cod liver oil, and two were kept on table scraps. In none of these animals were signs of the paralysis observed clinically. In one animal from a mother on 100 per cent calf-meal during pregnancy and lactation, and in two rats given the table scrap diet, a complete investigation of the nervous system, central and peripheral, by the histological methods used in this study, failed to reveal any degeneration. The fat content of the purified diet had apparently no deleterious effect on the nervous system.

It is evident that both the purified diet when supplemented with cod liver oil and the table scrap diet when given over a long period of time were capable of maintaining the rats in a satisfactory state of nutrition, without clinical or microscopic lesions of the nervous system.

PATHOLOGY

The gross lesions found at autopsy in the various groups were as follows: of the twelve rats in group I, six showed foci of infection in the kidneys; seven showed bladders filled with

pus; two showed lung infections; six showed infections of the glands at the base of the tongue. In group II the infection was less. Of ten animals examined there were found only one kidney lesion, two lung lesions and five infections of the tongue. Of six animals examined from group III, only one kidney and one tongue lesion were found. In groups IV and V no foci of infection were found. No salivary gland lesions were found on gross examination in any of the rats. Many of the tissues were checked by histological examination. No lesions were found on section of nine intestines, four stomachs, three livers, all taken from the most depleted animals in groups I and II. Infection was found in the tracheal glands of eight of the experimental animals examined. Necrosis was found in the vagina of three rats out of a total of fifteen examined histologically. The bone marrow from seven of the most depleted rats was not histologically different from that of the control.

DISCUSSION

In the group of rats dealt with in this experiment certain neurological symptoms with lesions of the nervous system developed from a lack of adequate vitamin A. These appeared only in those animals suffering from a long continued deficiency. In the acute cases the animals died before the paralysis was manifested. In the group where twelve animals started with little or no stored vitamin (group I), nine of the animals which lived beyond the time when severe avitaminosis was present exhibited clinical symptoms of the paralysis. All these rats showed evidence of neurological lesions.

The fifteen rats which started the experiment with some storage of vitamin A (group II) were heavier at weaning and throughout the experiment. Ten lived to show continual cornified smears, eight exhibited stage I or II, or both of the paralysis, and none had further symptoms of neural lesions. The first symptom of the paralysis appeared about 7 weeks later than the paralysis in the group which started the experiment without a storage of vitamin A. In the rats which

started the experiment without a storage of the vitamin the paralysis affected more animals and appeared more quickly, the symptoms were more pronounced, less time elapsed between symptoms, and death occurred sooner than in the rats which started the experiment with a storage of the vitamin. The symptoms of paralysis always occurred after the appearance of continual cornified vaginal cells and at about the same time or somewhat later than the xerophthalmia or loss of weight. The incidence, the severity, and the progression of the neural symptoms were shown to depend directly upon the amount of vitamin A available, either stored or ingested (Aberle, '31).

The paralysis did not appear in animals suffering severe inanition with plenty of vitamins (group IV), even though their weight approximated that of rats with severe avitaminosis. An examination of their nervous tissue confirmed the clinical diagnosis. At death the weight of the animals with a restricted diet averaged only 2 gm. less than that of the rats with stage II neural symptoms.

Anaemia was probably not a contributory factor. Turner ('30) has shown that rats on diets deficient in vitamin A do not show a difference in the non-protein nitrogen, creatinine, urea nitrogen, chloride, sugar, or red blood corpuscles when compared to the controls. In the bone marrow of rats examined microscopically, no difference could be ascertained between the experimental and control animals.

Although the symptoms resemble those described by Cowgill ('21) for dogs suffering from lack of vitamin B complex, yeast had no effect upon preventing, alleviating, or curing the disorder. All the rats received large daily feedings of yeast. In the seven animals where additional B was given in the form of concentrated yeast or tiki-tiki, no change either clinically or microscopically was noted.

Control rats on the purified diet with cod liver oil never developed neural disturbances clinically or histologically. Vitamin C was absent in both experimental and control groups, which would eliminate its importance as a factor re-

sponsible for the paralysis. The control rats were given the same proportions of the purified diet as were the experimental rats. Thus, the fat content of the diet was of no significance as far as the neural manifestations were concerned (Burr and Burr, '29). Four rats were cured clinically by administering cod liver oil. Their nervous tissue still showed lesions. When recovery takes place the tissue resembles tissue from the more acute pathological stages, but the physiology is different. In the opossum, Weed and Langworthy ('25) and Langworthy ('28) found that it was possible to stimulate the motor cortex and elicit movement in the fore legs 41 days before the cortico-spinal tract myelinated. Myelinization is not the exact criterion it has been supposed to be. It is not impossible that the axis cylinder may function before the myelin is redeposited. It might be that the axis cylinders in many cases were interrupted at a certain stage of avitaminosis and that later after resuming vitamin A feeding, the axis cylinder was again continuous.

Lesions of the central nervous system due to avitominosis of A are suggested by investigations dealing with dietary deficiencies which were reported some 19 years ago. Hart and McCollum ('14) reported the possibility of a 'toxic substance' in diets containing over 90 per cent of wheat. The ill effects of the diet were manifested in a loss of weight, rough coat, emaciation, lack of coordination of the muscles, and partial blindness. The conditions were found to be improved by the addition of butter fat.

Later, Hart, Miller and McCollum ('16) fed swine a diet low in vitamin A. After 9 months, growth ceased, followed by loss of weight, difficulty in locomotion, rough coat, labored breathing and muscular twitching. In some animals extreme stiffness and dragging of the rear quarters was evident. The investigators came to the conclusion that "malnutrition, histologically characterized by nerve degeneration, may result from the absence of certain factors in the diet as in the case of beri-beri. A similar condition may likewise arise from the presence of toxic materials in apparently normal food products . . . ."

Mellanby ('26, '30, '31) reported experiments in which puppies fed a diet deficient in fat soluble vitamins developed symptoms associated with nerve lesions. The most outstanding symptoms were severe incoordination, spasticity and weakness. The spinal cord of the animals, stained by Marchi's method, showed a scattered degeneration of the nerve fibers. Mellanby ascribes the condition to the 'toxic' effect of a large amount of cereal, other than yellow corn, in the diet.

On the other hand, Steenbock, Nelson and Hart ('21, '22) describe a peculiar gait in dogs on a diet deficient in vitamin A. No description is given of the lesions. Hughes et al. ('28, '29) reported nerve lesions in pigs, chickens and cows, due to lack of vitamin A. The symptoms were characterized by impaired vision, incoordination and spasms. They also describe the arching of the pig's back while standing or walking. This condition was noted in most of the rats and described under stage I of the paralysis. Histological examination of the nerves of the pigs in advanced stages of avitaminosis showed degeneration of the nerve bundles in portions of the spinal cord, and in the optic, sciatic and femoral nerves. No 'toxicity' is postulated for these experimental lesions. Suzman, Muller and Ungley ('32) attempted without success to produce lesions in the spinal cords of adult dogs by feeding a diet abundant in cereals but lacking in vitamin A. Their failure to obtain symptoms of avitaminosis was probably due to the age of the animals and the amount of stored vitamin with which they started the experiment. The lesions described by all of these investigators are somewhat similar and both clinically and histologically resemble those found in the rats here reported, clumsiness, incoordination, weakness and later on spasticity being the outstanding symptoms. In this experiment no cereal was fed. Before 'toxicity' of certain foods could be postulated, it would be necessary to feed animals on a diet adequate in vitamin A.

CONCLUSIONS

A disabling paralysis has appeared in rats suffering from chronic deficiency of vitamin A. The paralysis was characterized clinically by clumsiness, incoordination and finally a spasticity, with a complete lack of control of the affected limbs. Microscopical examination showed the paralysis to be associated with degeneration of parts of the nervous system.

The paralysis did not appear in control animals kept under identical experimental conditions, and fed the same purified foods, except for the inclusion of cod liver oil. It did not appear in rats on a table scrap diet, or in rats with severe inanition but provided with plenty of vitamins.

The paralysis was closely associated with other symptoms of avitaminosis. Neurological symptoms were always preceded by the continuous appearance of cornified vaginal cells, and were frequently preceded by xerophthalmia and loss of weight.

The incidence of paralysis among rats which at the beginning of the experiment were without stored vitamin A was greater, they exhibited the paralysis sooner, they had more pronounced symptoms, and they showed less time between symptoms and died sooner than rats which had come from parents whose vitamin A storage had been ample.

The writer is indebted to the Department of Physiological Chemistry, Yale University School of Medicine, for advice and suggestions in carrying out this investigation and to L. Brill for her help in the care and feeding of the animals.

LITERATURE CITED

ABERLE, S. B. D. 1933 Continual cornified vaginal cells as an index of avitaminosis-A in rats. J. Nutrition, vol. 6, p. 1.

ABERLE, S. B. D., H. M. ZIMMERMAN, AND L. BRILL 1931 Paralysis in rats on a vitamin-A low diet. Anat. Rec., vol. 48 (abstract), p. 7.

BURR, G. O., AND M. M. BURR 1929 A new deficiency disease produced by the rigid exclusions of fat from the diet. J. Biol. Chem., vol. 82, p. 345.

COWGILL, G. R. 1921 A contribution to the study of the relation between vitamin B and the nutrition of the dog. Am. J. Physiol., vol. 57, p. 420.

EVANS, H. M., AND G. O. BURR 1927 The antisterility vitamin fat soluble E. Memoirs Univ. California, vol. 8, p. 1.

HART, E. B., AND E. V. MCCOLLUM 1914 Influence on growth of rations restricted to the corn or wheat grain. J. Biol. Chem., vol. 19, p. 373.

HART, E. B., W. S. MILLER, AND E. V. MCCOLLUM 1916 Further studies on the nutritive deficiencies of wheat and grain mixtures and the pathological conditions produced in swine by their use. J. Biol. Chem., vol. 25, p. 239.

HESS, A. F. 1927 Antirachitic activity of irradiated cholesterol, ergosterol and allied substances. J. Am. Med. Assoc., vol. 89, p. 337.

HUGHES, J. S., C. E. AUBEL, AND H. F. LIENHARDT 1928 The importance of vitamin A and vitamin C in the ration of swine, concerning especially their effect on growth and reproduction. Kansas Agri. Sta. Tech. Bull., vol. 23, p. 1.

HUGHES, J. S., H. F. LIENHARDT, AND C. E. AUBEL 1929 Nerve degeneration resulting from avitaminosis-A J. Nutrition, vol. 2, p. 183.

LANGWORTHY, O. R. 1928 The behavior of pouch-young opossums correlated with the myelinization of tracts in the nervous system. J. Comp. Neur., vol. 46, p. 201.

MELLANBY, E. 1926 The presence in foodstuffs of substances having specific harmful effects under certain conditions. J. Physiol., vol. 61, proc. xxiv.

———— 1930 The relation of diet to health and disease. Brit. Med. J., vol. 1, p. 677.

———— 1931 Diseases produced and prevented by certain food constituents. J. Am. Med. Assoc., vol. 96, p. 325.

PARSONS, H. T. 1920 The antiscorbutic content of certain body tissues of the rat. J. Biol. Chem., vol. 44, p. 587.

STEENBOCK, H., E. M. NELSON, AND E. B. HART 1921–1922 Fat soluble vitamine. IX. The incidence of an ophthalmic reaction in dogs fed a fat soluble vitamine deficient diet. Am. J. Physiol., vol. 58, p. 14.

SUZMAN, M. M., G. L. MULLER, AND C. C. UNGLEY 1932 An attempt to produce spinal cord degeneration in dogs fed a high cereal diet deficient in vitamin A. Am. J. Physiol., vol. 101, p. 529.

TURNER, R. G. 1930 Blood findings in albino rats suffering from lack of vitamin A. Proc. Soc. Exper. Biol. and Med., vol. 27, p. 1006.

WEED, L. H., AND O. R. LANGWORTHY 1925 Developmental study of excitatory areas in the cerebral cortex of the opossum. Am. J. Physiol., vol. 72, pp. 8–24.

ZIMMERMAN, H. M. 1933 Lesions of the nervous system in vitamin deficiency. I. Rats on a diet low in vitamin A. J. Exper. Med., vol. 57, p. 215.

PLATE 1

EXPLANATION OF FIGURES

1 Control rat, showing characteristic flexion of the hind legs when held by the loose skin at the nape of the neck.

2 Rat showing stage I of the paralysis; a hyperextension of the hind legs and marked curvature of the spine when held by the loose skin at the nape of the neck. Nineteen days following the first symptom of vitamin A deficiency and 58 days on purified diet.

3 Rat showing stage II of the paralysis. The left foot slipped when walking. Nineteen days after first symptom of vitamin A deficiency and 58 days on purified diet.

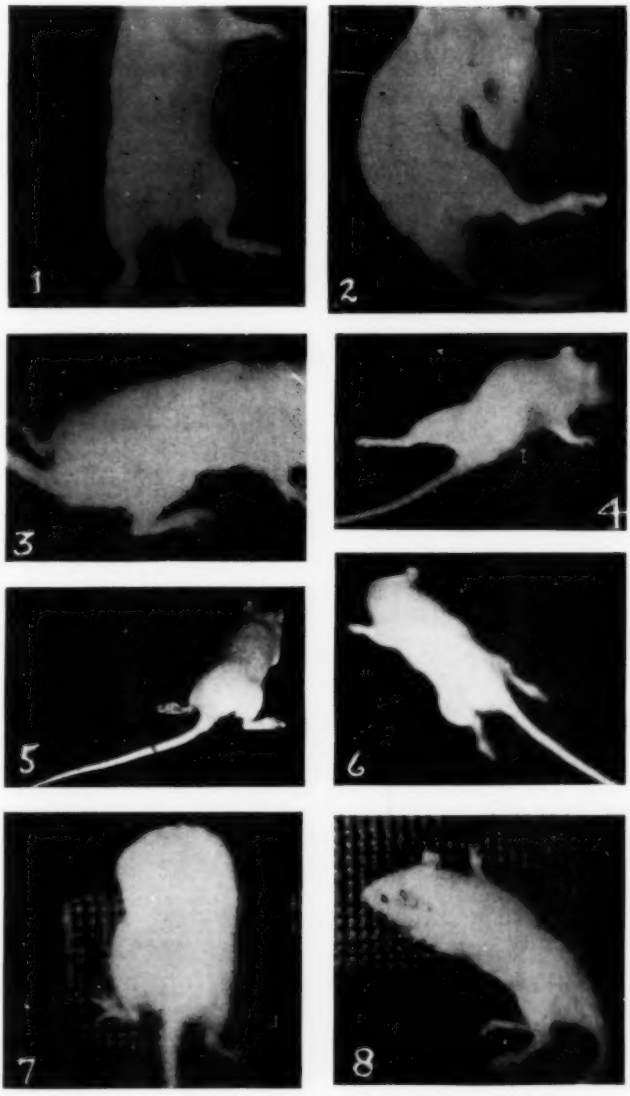
4 Rat showing stage II of the paralysis. The left leg has slipped away from the body, the right leg is beneath the body of the rat. Ten days after the first symptom of vitamin A deficiency and 45 days on purified diet.

5 Rat showing stage III of the paralysis. The hind legs are held out from the body and the feet are at about a 45° angle from the long axis of the rat. Firty-three days on purified diet and 18 days after first symptoms of vitamin A deficiency.

6 Rat showing stage IV of the paralysis. The front legs are used for locomotion while the hind legs are drawn helplessly along. Forty-three days on purified diet and 13 days after first symptom.

7 Control rat climbing over a wire fence. The rat had been 95 days on purified diet with cod liver oil.

8 Rat attempting to climb wire fence. Animal has been on the purified diets for 53 days, 18 days after the first symptom of vitamin A deficiency.


1
2
3
4
5
6
7
8

RELATION BETWEEN THE PHYSICAL CHARACTER OF FOOD AND DENTAL CARIES IN ALBINO RATS

C. A. LILLY AND LEONA WILEY

Department of Internal Medicine, Medical School, University of Michigan, Ann Arbor

TWO FIGURES

(Received for publication June 19, 1933)

There is no agreement as to the etiology of dental caries. Excess of carbohydrate, Bacillus acidophilus, deficiencies of vitamins A, B, C, and D, as well as minerals have all been suggested as causative factors. More recently the physical character of the dietary constituents has been accused.

We have reported that different diets containing 73 per cent of starch, 66 per cent of glucose, 66 per cent of lactose and 66 per cent of maltose failed to produce dental caries in 6½ months (Lilly and Grace, '32) and that triweekly inoculations with the Bacillus acidophilus of the mouths of rats for a year was not provocative of dental caries even in animals subsisting on high carbohydrate diets for a year (Lilly, '32). We also attempted to produce dental caries by placing rats on diets deficient in vitamin D. These animals were kept in a dark room on Steenbock's Rachitiogenic Diet no. 2965 and distilled water, and received no added vitamin D. Some of these animals lived 6 and 7 months and two survived 11 and 12 months respectively, yet no caries developed in any of these animals.

In addition, avitaminosis and slow starvation produced no caries in rats under our observations, since Mendel's normal rat diet made up with deficiencies of either vitamins A, B or

D (rats synthesize vitamin C) produced no caries up to the death of those animals. Furthermore, other diets so low in protein and minerals that only one-half the size and weight and one-fourth the reproduction of normal rats were attained, produced no dental caries in three successive generations.

In several hundred autopsies on rats fed either on table scraps or Mendel's normal rat ration, in this laboratory, not a single instance of dental caries has occurred.

These observations made it evident that there was nothing inherent in the routine management of our animals that produced dental caries.

The experiment about to be reported deals with the idea suggested by Hoppert, Webber and Canniff ('31, '32) who report the occurrence of dental caries in relation to 'particle size' and 'retention particles' of cornmeal-fed albino rats.

Klein and McCollum ('31), in a comment, attributed the caries reported by Hoppert, Webber and Canniff to a phosphorus deficiency of the caries producing diet.

The basic diet used in our experiment was as follows:

	Per cent
Yellow cornmeal, ...	60
Whole dry milk[1]	30
Flaxseed meal	6
Alfalfa meal	3
NaCl	1
	100

[1] Klim.

This is a stock ration used by many investigators for normal rat colonies.

To test the efficiency of this ration on our strain of rats, we fed a colony of our animals this diet through four generations and found it maintained an average of size, weight, and reproduction comparable to that produced by Mendel's normal rat diet.

This diet having proved adequate according to present standards was then varied as follows; however, one variable only was permitted in each test.

Series I. The basic diet was made up by using yellow cornmeal from a nearby grocery store. This was a fine meal, as commercial meal is screened before being put on the market.

Thirty-five rats, 26 to 31 days old, from our own colony were fed this diet for 200 days. The animals were then chloroformed, the heads cleaned, and the teeth examined under magnification but no caries was present.

Series II. Yellow corn was then coarsely ground in a cornmeal mill, and the whole product collected. This product was then sifted in a 60-mesh standard sieve; about one-third of the meal passed through the sieve, while about two-thirds did not. Thus a fine and a coarse meal was obtained.[2]

Group 'A.' The basic diet was then prepared by using the meal that did not pass the sieve.

Twenty-four rats, 27 to 30 days old, from our own rat colony were fed this diet for 100 days. The animals were then chloroformed, the heads cleaned, and the teeth examined under magnification for caries when fifteen of the twenty-four rats or 62 per cent were found to have from one to three large carious lesions in the molar teeth.

Group 'B.' The basic diet was prepared by using the meal that passed the sieve.

Twenty-four rats, 27 to 30 days old, from our own colony were fed this diet for 100 days. The animals were then chloroformed, the heads cleaned and the teeth examined under magnification for caries but no caries was found.

Group 'C.' The basic diet of this group was identical to the diet fed group A, except that the meal was ground through a 'hammer mill' before being mixed with the other ingredients of the diet. The hammer mill[3] reduced the meal only sufficiently for two-thirds of it to pass the 60-mesh sieve.

Twenty-four rats, 25 to 30 days old, from our own colony were fed this diet for 100 days, when the animals were chloro-

[2] The sifting is a tedious process, a stiff brush being needed to express the fine portion through the sieve.

[3] We are indebted to the Chelsea Flour Milling Company for the use of the hammer mill.

formed, the heads cleaned, and the teeth examined under magnification for caries. Five of the twenty-four rats (or 20.8 per cent) had one or more caries.

Group 'D.' The basic diet of this group was identical to that fed group 'A' except that $\frac{3}{10}$ per cent viosterol (250 D) by weight was incorporated into the diet.

Fifteen rats, 25 to 30 days old, from our colony were fed this diet for 100 days, then chloroformed, the heads cleaned, and the teeth examined under magnification for caries. Eight of these fifteen rats (or 53 per cent) had one or more dental caries.

Series III. A new lot of yellow corn was coarsely ground and the whole product collected and sifted in a 60-mesh sieve and a fine and a coarse meal obtained exactly as in series II.

Group 'A.' The basic diet was prepared by using the meal that did not pass the 60-mesh sieve.

Sixteen rats, 25 to 30 days old, from our own colony were fed this diet for 125 days (25 days longer than in series II). The animals were then chloroformed, the heads cleaned and the teeth examined under magnification for dental caries. All of the animals (100 per cent) had one to three large carious molar teeth.

Group 'B.' The basic diet was prepared by using the meal that passed the 60-mesh sieve.

Ten rats, 25 to 30 days old, from our own colony were fed this diet for 125 days. The animals then were chloroformed and examined for dental caries as previously described but no caries was present.

Series IV. A new lot of yellow corn was coarsely ground, the whole product collected, sifted in a 60-mesh standard sieve, and a fine and a coarse meal obtained exactly as in series II and III.

Group 'A.' The basic diet was prepared by using the meal that did not pass the 60-mesh sieve.

Nine rats,[4] 25 to 30 days, old, from our own colony were fed this diet for 100 days. The animals were then chloro-

[4] This group was fed as a 'control' for this lot of corn, and found productive of caries.

formed and examined as previously described for dental caries.[5]

Four of the nine rats (or 44 per cent) had one or more carious molar teeth.

Group 'B.' The diet for this group was identical to the diet fed group 'A' except that the meal was cooked for 2½ hours in a double boiler under distilled water before it was mixed with the other ingredients of the diet.

Sixteen rats, 25 to 30 days old, from our own rat colony were then fed this diet for 100 days, then chloroformed and examined as previously described for dental caries but no caries was present.

Group 'C.' The diet for this group was identical to the diet of group 'A,' except that the meal was ground through a Schultz-O'Neil mill[6] before it was mixed with the other ingredients of the diet.

The Schultz-O'Neil mill ground the meal fine enough to pass a 60-mesh sieve. However, a very small portion of a chaffy material did not pass the sieve; this was reduced by mortar and pestle until it did, then put back into the diet.

Sixteen rats, 25 to 30 days old, from our own colony were fed this diet 100 days, then chloroformed and examined for dental caries as previously described but no caries was present.

Calcium and phosphorus metabolism

Calcium and phosphorus balances were obtained for 5-day periods during the preceding experiments for the finely ground corn and the coarse meal, both cooked and uncooked. In each experiment the rats were placed together in a large cage and the food carefully weighed. At the end of the experiment the cage was carefully cleaned and washed, all of the excreta and washings being placed in a large round-bottomed flask. Any food which had been dropped in the cage

[5] Ten animals were started on this test; one died at the end of the first month of the test.

[6] We are indebted to Frederick Stearns & Co., of Detroit, Michigan, for the use of their Schultz-O'Neil mill.

was included with the material in the flask. The contents of the flask were evaporated to dryness in an oven, and digested for several hours with concentrated hydrochloric acid under a reflux condenser. The material was then centrifuged and washed three times with water, and the liquid obtained by centrifuging and the washings was placed in a 2-liter volumetric flask. The precipitate was returned to the digestion

Fig. 1 Microphotograph of a typical dental caries in a lower molar of an albino rat fed on the coarse cornmeal diet for 100 days.

Fig. 2 Enlarged photograph of lower jaw of rat fed the coarse cornmeal diet for 100 days, showing typical dental caries.

flask and the process repeated until the precipitate in the centrifuge tubes gave no test for calcium or phosphorus. The combined liquors and washings were made up to volume in the 2-liter flask.

Aliquot parts of this solution and the diets were analyzed for calcium by the method of Tisdall and Kramer ('21) and for total phosphorus by the method of Fiske and Subbarow ('25).

The results of these experiments are shown in table 1. Since the food which was lost in the cage was included with

TABLE 1

Calcium and phosphorus balances

DIET	NUMBER OF RATS	CALCIUM			PHOSPHORUS		
		In	Out	Balance	In	Out	Balance
		mg.	*mg.*	*mg./rat/day*	*mg.*	*mg.*	*mg./rat/day*
Coarse	7	1285	899	+11.3	1750	1250	+14.3
Coarse	3	655	576	+ 5.3	883	714	+11.3
Coarse cooked	7	1260	1220	+ 1.1	1670	1620	+ 1.4
Fine	6	1089	939	+ 5.0	1245	1110	+ 4.5
Fine	1	245	232	+ 2.6	268	217	+10.2

the excreta in the determination of calcium and phosphorus, this factor does not influence the estimation of the balance for these two elements. The balances were calculated per rat per day. Two experiments on the coarse uncooked diet gave calcium retentions of 11.3 and 5.3 mg. and phosphorus retentions of 14.3 and 11.3 mg. respectively. One experiment on the cooked coarse diet indicated that the rats were practically in calcium balance. Two experiments on the fine diet gave calcium retentions of 5.0 and 2.6 mg. and phosphorus retentions of 4.5 and 10.2 mg. respectively. From these results it would seem that the appearance of dental caries under these conditions is not due to the inability of the animal to

maintain calcium and phosphorus balance, since the greatest retention of these two elements was found in the rats which showed the greatest incidence of caries.

Group 'D.' The diet of this group was identical to the diet fed group 'C' except that 1 per cent of the weight of the diet in the form of washed, ignited sea sand was added to and mixed through the diet. The mesh of the sand was determined by, first, discarding all that portion of the sand that did not pass a 40-mesh standard sieve, and second, by then discarding all that portion that did pass a 60-mesh standard sieve.

Group 'E.' The diet of this group was identical to the diet fed group 'C' except that 5 per cent of the weight of the diet in the form of washed ignited sea sand was added to and mixed through the diet.

Twelve rats, 25 to 30 days old, from our own rat colony were fed this diet for 100 days, then chloroformed and examined for dental caries but no caries was found.

Group 'F.' The diet of this group was identical to the diet fed group 'C' except that 10 per cent of the weight of the diet in the form of washed ignited sea sand was added to and mixed through the diet.

Twelve rats, from 25 to 30 days old, from our own rat colony were fed this diet for 100 days, then chloroformed and examined for dental caries. It was found that three of these twelve rats (25 per cent) had one or more carious molar teeth.

DISCUSSION

Since the occurrence of caries, or its absence, was determined by feeding precisely the same diet in the uncooked state, or after it had been softened by cooking, the occurrence of the caries is not attributable to chemical differences in the two instances.

Furthermore, since caries could not be produced or prevented by feeding the same material in a coarse state or after it had been reduced to a fine powder, it is again clear that the caries we produced could not be directly related to the chemical constitution of the diet.

It should also be pointed out that the rats that developed caries retained more calcium and phosphorus than the rats that avoided caries.

It seems clear that the physical form of the diet was the predisposing factor of the occurrence of the caries. Since the particles of coarse cornmeal are exceedingly hard, it is conceivable that the animals fractured the enamel of their teeth as a result of chewing this material and that this initiated a destructive process.

It is, however, no clear how such a destructive process would take place, whether by digestion, the result of bacterial invasion, or failure of repair.

Another possible explanation appears to be worth mentioning, namely, that the particles of corn became wedged between opposing teeth, and that condition initiated any one of the three processes mentioned above.

We have however at the present no satisfactory explanation for the occurrence of the caries. We have nothing to offer other than the outstanding fact that coarse hard particles of cornmeal in the diet regularly caused the teeth of rats that ate this diet for 3 or 4 months to become carious to a high degree.

CONCLUSION

Diets adequate for growth, weight, and reproduction according to accepted standards, containing coarse cornmeal produced dental caries in 66 per cent of albino rats in 100 days and in 100 per cent of rats in 125 days.

The incidence of caries was related to the physical form of the food (cornmeal) and not to the calcium or phosphorus values of the diet.

If the coarse cornmeal were made soft by cooking or ground fine enough to pass a 60-mesh sieve no caries was produced. Vitamin D did not prevent the occurrence of the caries.

We wish to express our appreciation of the advice given us by Dr. L. H. Newburgh during this test.

LITERATURE CITED

FISKE, C. F., AND Y. SUBBAROW 1925 The calorimetric determination of phosphorus. J. Biol. Chem., vol. 66, p. 373.

HOPPERT, C. A., P. A. WEBBER AND T. L. CANNIFF 1931 The production of dental caries in rats fed adequate diets. Science, vol. 74, p. 77.

———— 1932 Production of dental caries in rats fed an adequate diet. J. Dent. Res., vol. 12, p. 161.

KLEIN, HENRY, AND E. V. MCCOLLUM 1931 A preliminary note on the significance of the phosphorus intake in the diet and blood phosphorus concentration, in the experimental production of caries-immunity and caries susceptibility in the rat. Science, vol. 74, p. 662.

LILLY, C. A., AND J. D. GRACE 1932 Failure to produce dental caries with high carbohydrate and with low fat diets. Proc. Soc. Exp. Biol. and Med., vol. 29, p. 176.

LILLY, C. A. 1932 Failure to produce dental caries in the white rat with high carbohydrate diet and Bacillus acidophilus or with vitamin D deficiency. J. Nutrition, vol. 5, p. 175.

TISDALL, F. F., AND B. KRAMER 1921 Methods for the direct quantitative determination of sodium, potassium, calcium and magnesium in urine and stools. J. Biol. Chem., vol. 48, p. 1.

ANALYSES OF MEATS

V. A. TOSCANI, V. R. RUPP AND W. S. McCLELLAN
The Russell Sage Institute of Pathology in affiliation with the Second Medical (Cornell) Division of Bellevue Hospital, New York City

(Received for publication June 27, 1933)

During the course of an investigation of the metabolism of two men who received an exclusive meat diet for 1 year, a series of determinations were made on the various cuts of meats which duplicated those consumed by the two men. A résumé of the observations of their metabolism was reported by McClellan and DuBois ('30), McClellan, Rupp and Toscani ('30) and McClellan, Spencer and Falk ('31). Sherman ('32) gives a detailed analysis of the edible organic nutrients, in which he differentiates between various cuts of the same foodstuff. In our investigation no attempt was made to sort the various cuts of beef muscle, the primary object being to determine the actual intake by the patients of the various organic and ash constituents. In this study the only source of carbohydrate was contained in the meat consumed by the subjects. The published tables (Sherman, '32) omit carbohydrate in their analyses, as the amount of carbohydrate contained in a portion of meat in a mixed diet is not significant.

This paper contains the values for protein, fat, carbohydrate, calcium and phosphorus as reported by McClellan, Rupp and Toscani ('30). The purpose of this report is to present a complete table on the meats analyzed including the total ash and water content.

PREPARATION OF MATERIAL

During the first 3 months the meat used was obtained from animals slaughtered the preceding day. After the third

month, ordinary refrigerated meats were used. Meals which duplicated as closely as possible those eaten by the men were sent to the laboratory daily in enameled pails and preserved on ice for the length of a period, which was usually from 7 to 10 days. The meat was then sorted, weighed, ground in a meat chopper, and dried on a steam bath in porcelain evaporating dishes. When dry, the specimens were weighed, ground and preserved in Mason jars for subsequent analysis. An aliquot portion of the stored sample was finely ground in a porcelain mortar, quartered and samples taken for duplicate analysis.

METHODS

Nitrogen

Duplicate samples of 0.5 gm. of the dried material were digested with 20 cc. of an acid mixture[1] in 500-cc. Kjeldahl flasks. The flasks were heated slowly until carbonization was complete; then the digestion was allowed to proceed at a more rapid rate until no particles of carbon were visible. The regular Kjeldahl procedure was followed from this point. The values for protein were obtained by multiplying the nitrogen in grams by 6.25.

Fat

Duplicate analyses were made on 5.0-gm. portions of the dried meats. The fat was extracted with anhydrous ether overnight, using the Soxhlet apparatus. The ether was recovered by distillation on a warm water bath. The fat residue in the Soxhlet flask was then extracted with redistilled petroleum ether, using the fraction which distilled over between 40° and 60°C. The fat containing ether was filtered through non-absorbent cotton into a Petri dish. The flask and funnel were washed several times with small quantities of petroleum

[1] The acid mixture consists of the following reagents:

5 per cent copper sulphate	50 cc.
85 per cent phosphoric acid	300 cc.
Concentrated sulphuric acid	100 cc.

ether to insure maximum recovery. The Petri dishes were then placed on a warm surface to permit the evaporation of the ether. When most of the ether had evaporated, the dishes were placed on a hot surface to remove the last traces of the solvent. After cooling, the extracted fat was weighed.

Carbohydrate

Hydrolyzable fraction. For this determination 0.5 gm. of the sample of dried meat was ground in a mixture of white sand and 2.2 per cent hydrochloric acid and made up to 50 cc. with the 2.2 per cent acid in a potato tube. The mixture was hydrolyzed for 5 hours in a boiling water bath. The hydrolyzed sample was neutralized with 50 per cent sodium hydroxide using litmus paper as the indicator. Four grams of trichloracetic acid were added and the mixture was allowed to stand for 1 hour and then made up to 100 cc. with distilled water. The material was filtered, made up to volume and an aliquot portion taken for analysis. Determinations of the reagent blanks were made at the same time. The hydrolyzable fraction of the carbohydrate was determined by the procedure of Hagedorn and Jensen ('23). The hydrolyzed aliquot sample was made alkaline with 0.1 N sodium hydroxide using phenol red as indicator. Two cubic centimeters of 0.005 N potassium ferricyanide were added and the mixture boiled for 15 minutes in a boiling water bath. The tubes were allowed to cool to room temperature and 3 cc. of iodide mixture,[2] 2 cc. of 3 per cent acetic acid and 5 drops of starch were added. The solution was titrated against 0.005 N thiosulphate, using a microburette graduated in 0.01 cc. As the usual starch solution was not as sensitive as desired, starch prepared in the following way gave delicate end points. One gram of soluble starch was ground to a paste with a small

[2] The iodide mixture consists of the following reagents:

Zinc sulphate	10 gm.
Potassium iodide	5 gm.
Sodium chloride	50 gm.
Made up in 200 cc. of distilled water	

quantity of saturated sodium chloride solution. The pasty mass was made up to 100 cc. with hot saturated salt solution. This salt solution of starch, when well stoppered, kept for a long period of time.

Lactic acid. One gram of the dried meat was ground in sand and 4 per cent trichloracetic acid, transferred to a 100-cc. measuring cylinder, made up to the mark with the 4 per cent acid and allowed to stand for 1 hour. The mixture was filtered into a 200-cc. stoppered measuring cylinder. Ninety cubic centimeters of the filtrate were neutralized with 50 per cent sodium hydroxide using brom-cresol-green as indicator. To the neutralized filtrate 40 cc. of 20 per cent copper sulphate and 40 cc. of a 10 per cent suspension of calcium hydroxide were added and made up to the mark with distilled water and shaken. The mixture was allowed to stand for 1 hour and then filtered through a dry paper. Twenty cubic centimeters of the filtrate were used for each determination. The procedure of Friedemann, Cotonio and Shaffer ('27) was followed from this stage.

Total carbohydrate. This was obtained by adding the values for the lactic acid content and the hydrolyzable fraction. It affords a fairly accurate measure of the total carbohydrate content of tissue. A small carbohydrate fraction, which is not susceptible to complete hydrolysis, is left undetermined by this method, but it is probably fairly constant under the conditions of the experiment and not significant in amount.

Calcium

Calcium was determined by McCrudden's method ('11, '12) on 10.0 gm. of the dried material after ashing.

Phosphorus

Phosphorus was estimated on 1.0-gm. portions of the dried samples by the volumetric molybdate method (Official and tentative methods of analysis of the Association of Official Agricultural Chemists, '25) after digestion with nitric and sulphuric acids.

Ash

The total ash was determined by ashing 10.0-gm. samples of the dried meats in porcelain crucibles. Samples that ashed with difficulty were treated with a few drops of nitric acid. Specimens that did not ash satisfactorily with the latter treatment were ashed by passing a slow stream of oxygen over the hot ash.

Water

The difference between the sum of the amounts of protein, fat, carbyohydrate and total ash, and 100 was considered to be the amount of water present in 100 gm. of the fresh meat.

DISCUSSION

The results of the analyses which were made are presented in the table. Only the maximum, minimum and average values are included. There was close agreement between the values for protein obtained in this series and those reported in the standard tables.

The amount of fat in any cut of meat is a variable factor. Even in beef muscle which was trimmed as carefully as possible to remove all excess fat, variations in the fat content occurred between 2.9 and 7.6 per cent. This was due to differences in the amount of fat lying between the strands and fibers of the muscle which could not be removed by trimming. The amount of fat of this nature is dependent on the food with which the animal was fatted. In beef tongue, the variability in its fat content depended largely on the section of tongue used for the analysis. As these samples of meat were duplicates of those served in diets, sometimes there was a larger amount from the posterior portion of the tongue which was the section containing the greatest amount of fat and sometimes more from the anterior, lean portion.

In the standard food tables, the amount of carbohydrate in meat is entirely neglected and no values are given except for liver. In the studies for which these analyses were made, the extremely low carbohydrate intake made it important to

TABLE 1

Analysis of meats. Grams per 100 gm. moist weight

NATURE OF MEAT	NUMBER OF SAMPLES	PROTEIN			FAT			CARBOHYDRATE			CALCIUM		
		Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Beef muscle, well trimmed	24	21.80	18.15	20.19	7.60	2.88	5.60	1.49	1.35	1.36	0.0152	0.0072	0.0144
Beef muscle, not trimmed	10	22.50	17.75	19.85	20.60	7.90	13.13	1.38	1.12	1.32	0.0169	0.0077	0.0119
Beef tongue	19	26.00	16.90	19.85	34.60	8.00	23.60	1.53	0.72	1.09	0.0150	0.0063	0.0081
Beef liver	14	24.30	15.30	20.20	8.55	4.20	6.34	3.47	1.70	2.52	0.0120	0.0056	0.0077
Beef kidney	2	15.40	13.90	14.65	2.50	2.40	2.45	0.40	0.40	0.40	0.0070	0.0070	0.0070
Beef brain	1			10.50			9.00			1.08			0.0077
Lamb muscle	4	16.00	13.95	15.14	33.60	22.90	27.00	0.76	0.54	0.69	0.0327	0.0109	0.0210
Veal muscle	3	18.80	17.20	17.80	17.20	13.50	15.96	1.62	1.38	1.50	0.0155	0.0134	0.0145

NATURE OF MEAT	NUMBER OF SAMPLES	PHOSPHORUS			ASH			WATER		
		Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Beef muscle, well trimmed	24	0.236	0.204	0.220	1.38	0.89	1.06	74.9	69.8	71.9
Beef muscle, not trimmed	10	0.260	0.194	0.222	1.23	0.85	1.01	69.9	54.4	65.7
Beef tongue	19	0.260	0.160	0.199	1.18	0.64	0.89	68.0	42.9	54.5
Beef liver	14	0.480	0.310	0.420	1.91	1.35	1.70	75.5	65.8	69.2
Beef kidney	2	0.252	0.252	0.252	1.10	1.06	1.08	82.1	80.7	81.4
Beef brain	1			0.380			1.61			77.8
Lamb muscle	4	0.189	0.170	0.180	0.99	0.77	0.85	59.7	49.9	56.4
Veal muscle	3	0.261	0.191	0.223	1.10	0.86	0.96	65.5	62.8	63.8

take into account even small amounts of carbohydrates. In order to include all material which might exert any antiketogenic effect, the total carbohydrates were taken as the sum of hydrolyzable carbohydrate and lactic acid as determined in the analyses. On the basis of this computation it was found that the men were taking 8 to 12 gm. of carbohydrate per day in their diets.

The determinations of minerals such as calcium and phosphorus and the total ash are of importance and emphasize particularly the low calcium content in meat. The higher values for phosphorus in liver and kidney as compared with the amounts in other cuts are well known.

The values for water only stress again the large amount of this constituent in many of the foods in the normal diet and the absolute necessity of considering the water in food in any studies regarding the water balance.

SUMMARY

1. Analyses are reported for seventy-seven samples of meat. They include beef muscle, tongue, liver, kidney and brain. A few samples of lamb and veal muscle were analyzed. The significant features of these analyses have been considered in the previous discussion.

2. In order to have a good check on the values of the various foodstuffs consumed when meat makes up a considerable portion of the diet to be studied, it is important to make analyses of samples of meat which duplicate as closely as possible the meat eaten.

Lamb muscle	4	0.189	0.170						
Veal muscle	3	0.261	0.191	0.223	1.10	0.86	0.96	65.5	62.8	63.8

LITERATURE CITED

FRIEDEMANN, T. E., M. COTONIO, AND P. A. SHAFFER 1927 The determination of lactic acid. J. Biol. Chem., vol. 73, p. 335.

HAGEDORN, H. C., AND B. N. JENSEN 1923 Zur Microbestimmung des Blutzucker mittels Ferricyanid. Biochem. Zeit., Bd. 135, S. 46, and 1923 Die Ferricyanid methode zur Blutzuckerbestimmung. II. Biochem. Zeit., Bd. 137, S. 92.

MCCLELLAN, W. S., AND E. F. DUBOIS 1930 Clinical calorimetry. XLV. Prolonged meat diets with study of kidney function and ketosis. J. Biol. Chem., vol. 87, p. 651.

MCCLELLAN, W. S., V. RUPP AND V. A. TOSCANI 1930 Clinical calorimetry. XLVI. Prolonged meat diets with study of metabolism of nitrogen, calcium and phosphorus. J. Biol. Chem., vol. 87, p. 669.

MCCLELLAN, W. S., H. J. SPENCER AND E. FALK 1931 Clinical calorimetry. XLVII. Prolonged meat diets with study of respiratory metabolism. J. Biol. Chem., vol. 93, p. 419.

MCCRUDDEN, F. H. 1911–1912 The determination of calcium in the presence of magnesium and phosphates: the determination of calcium in the urine. J. Biol. Chem., vol. 10, p. 187.

Official and tentative methods of analysis of the Association of Official Agricultural Chemists, Washington, 1925, 2nd ed., p. 3.

SHERMAN, H. C. 1932 The chemistry of food and nutrition. The Macmillan Company, New York, 4th ed., p. 544.